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CORNELL UNIVERSITY MEDICAL BULLETIN

VOLUME X NUMBER 2

OCTOBER, 1920

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STUDIES FROM THE Department of PHARMACOLOGY

1919/20

PUBLISHED BY CORNELL UNIVERSITY
477 FIRST AVENUE
NEW YORK CITY

CORNELL UNIVERSITY MEDICAL BULLETIN

Issued Quarterly

Entered as Second Class Matter, June 23, 1911, at the Post Office, at New York, N. Y., under the Act of August 24, 1912

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized

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These publications include
The Annual Announcement of the Medical College and
Reprinted Studies from the Several Departments of the Medical College and its Research Laboratories

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1919-1920

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Reprinted from The Journal of Pharmacology and Experimental Therapeutics,
Vol. XII, No. 8, March, 1919

STUDIES IN THE ELIMINATION OF CERTAIN OF THE DIGITALIS BODIES FROM THE ANIMAL ORGANISM

ROBERT A. HATCHER and CARY EGGLESTON

From the Laboratory of Pharmacology of Cornell University Medical College, New York City

Received for publication January 24, 1919

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I. INTRODUCTION WITH DISCUSSION OF LITERATURE

Our knowledge of the elimination of the various digitalis principles from the animal organism is slight though the subject has been studied frequently. The lack of greater progress along this line is attributable to various causes among which is the want of suitable methods of recovering the active principles of this group from animal tissues and excreta in the form of pure solutions, and of estimating these principles quantitatively in impure solutions of tissue extracts.

Much confusion has resulted from applying the results obtained with one principle to others of the group, and from assuming that results obtained with one species of animal are applicable to others, and even to man, without determining the conditions that modify the actions of the substances in question. Even the mode of administration is of importance, for example, it is impossible to produce any perceptible effect in rats by oral doses of ouabain thousands of times larger relatively than would be fatal to the cat or dog by intravenous injection. Many of these modifying conditions have come to be so well recognized that one is prone to forget that they were not so well understood when much of the work still cited was done.

This condition has been further increased by the confusion in names of various digitalis bodies, for example, the word "digitalin" is commonly employed, but it is meaningless without a qualifying term. It has been used to mean digitoxin, true digitalin, or a mixture of the latter with digitonin, a saponin-like substance. So the word "strophanthin" is often used incorrectly for ouabain, as well as for amorphous strophanthins from different sources, of different degrees of activity and probably of different degrees of purity.[1] Much of the strophanthus of commerce was formerly of uncertain origin, and even careful investigators were frequently misled concerning the source of the strophanthus and strophanthin with which they worked,

[1] See New and Nonofficial Remedies, American Medical Association, 1918, p. 98, for a brief discussion of the important digitalis principles.

but it is now generally assumed that we can obtain the drug of known botanical source.

We are not convinced, however, that all commercial specimens of strophanthus—even those obtained from reputable dealers—are sold under their correct botanical names. One of us (1) has recently examined a specimen of strophanthus seed which had been purchased from a prominent drug miller who assured him that it was genuine *S. Kombe*. It yielded a fixed oil agreeing in amount and in physical properties with that obtained by Catillon (2) from a specimen of *S. hispidus*, the authenticity of which he believed to be beyond question. Another specimen of *S. Kombe* obtained from H. H. Rusby yielded an oil of quite different physical properties.[2]

The greater number of the experiments with amorphous strophanthin in the present research were carried out with the same specimens that were used by Hatcher and Bailey in 1909 and 1910. When possible we give the commercial source of the strophanthin with its supposed botanical origin, but we cannot vouch for the accuracy of the latter. We have recently (1918) examined these specimens by means of the biological test on cats and find that they have not undergone any appreciable change since they were purchased.

Crystalline ouabain was isolated from ouabaio wood about thirty years ago by Arnaud (3) who described it accurately and

[2] We have at no time concerned ourselves with the chemical investigation of the different digitalis principles, though Hatcher and Bailey stated, only parenthetically, on the basis of accepted investigations of others, that amorphous strophanthin is methyl ouabain (J. Am. Med. Assoc., 1910, lv, 1697). Brauns and Closson (J. Am. Pharm. Assoc., 1913, ii, 604) take pains to point out an assumed error on the part of those authors, supposing that they referred to the Kombe strophanthin alone. This is a pure assumption on their part, for it was stated distinctly in a previous paper (J. Am. Med. Assoc., 1909, lii, 5) that the strophanthin of Merck, and that of Boehringer and Sons were used, and it was then fairly well understood that Merck's strophanthin was made from *S. Kombe*, while Boehringer and Sons made theirs from *S. hispidus*. The American firm of Merck and Company was unable to tell us in January, 1916, whether the strophanthin obtained from them in 1908 by Hatcher and Bailey was made from *S. Kombe* or *S. hispidus*.

named it ouabain. He also established its identity with that of the principle obtained from *Strophanthus gratus* (then called *S. glabre* du Gabon), but some sixteen years later Thoms (4) rechristened it "crystalline strophanthin-g," thereby causing confusion.

In the present article we shall refer to this substance as ouabain, but in practically every instance it was called "strophanthin" or "crystalline strophanthin" by the various authors cited. Some authors who have used the amorphous strophanthin refer to it as "crystalline," and it is frequently impossible to determine whether the amorphous strophanthin used was from *S. hispidus* or *S. Kombe*, but this is probably of little importance, since they appear to be identical in behavior in the animal organism. There is a crystalline Kombe strophanthin but it is rarely employed in pure form.

The following references to the literature are intended to be suggestive, rather than comprehensive, and some of the papers are cited here because of the fact that they are often quoted in a way that renders them misleading, and we wish to point out the errors to which they lead. It is obviously impossible to escape all of the errors into which others have fallen, especially when attempting to explain statements that have proved misleading to others, but we trust that we can show the need of circumspection in accepting generalizations concerning the actions of drugs of this group.

It is not convenient to classify strictly researches dealing with the fate of the digitalis principles in the animal body, but for the most part these are concerned with their disappearance from the blood stream; their fixation or distribution in the tissues, especially in the heart and liver; their detection and estimation in the urine; their decomposition by acids, alkalies, the digestive ferments, and by bacteria; and their disappearance from the alimentary canal.

Koellicker, 1857, is said to have extracted antiarin from tissues and tested the extract biologically, at least Hedbom (5) states that he followed Koellicker's method and refers to Koellicker's paper on Upas Antiar (6) but we find no description of such

extraction or tests in that article, in which Koellicker stated that further work would be done.

Polaillon and Carville (7) state that the active principle of inée (strophanthus, almost certainly gratus, which contains ouabain), is not eliminated by the urine, saliva, or intestine of the frog, but that it accumulates in the blood. Their conclusion with reference to excretion is probably valid, but they used massive doses and it is not remarkable that they should have detected very small amounts in the blood of the frog. They also isolated enough of the poison from the blood of a dog to cause toxic symptoms in a frog. It is hardly justifiable to conclude from this that it, "accumulates" in the blood. Their results are commonly cited but they have little practical value in determining the fate of the digitalis bodies.

Hedbom (5) following the method of Koellicker—so he states—tried to extract antiarin from the hearts of severely poisoned frogs and to detect the substance by means of tests on the hearts of other frogs. His extracts exerted a weak action but he was unable to induce typical systolic standstill. His tests of the urine were negative.

Heuser (8) took advantage of the fact that the toad is resistant to the action of the digitalis principles to study the distribution of strophanthin (source not stated, probably the amorphous, from *S. Kombe*, then official in the German Pharmacopoeia), in the body. He found the toad's serum incapable of completely destroying strophanthin treated with it, and he was able to induce systolic standstill in *R. temporaria* with 1 cc. of the serum obtained from a toad that had previously had a dose of 2 mgm. of strophanthin. Since 2 mgm. are sufficient to kill about one hundred and fifty frogs of the size used for the test, the experiment can hardly be said to prove whether any destruction occurs or not. He could detect strophanthin in the intestine of the toad, a result which may be accepted.

Cloetta and Fischer (9) state that when digitoxin is shaken with a suspension of ground heart tissues and the mixture allowed to stand, the lower portion contains a relatively larger amount of the digitoxin than the supernatant fluid; liver and brain tissues

similarly treated do not carry down the digitoxin in combination. The authors worked with a secret preparation, known as digalen, which is said by Kiliani (10) to be nothing but a high percentage of digitalein, ("Das Digalen wird nichts anderes als hochprozentiges Digitalein"), but they make the amazing statement that their results are doubtless applicable to digitoxin.

Since digalen, which Cloetta arbitrarily called "amorphous" or "soluble" digitoxin, bears little resemblance to true digitoxin, the conclusions of Cloetta and Fisher have very little value and their assumption that the results are applicable to digitoxin has served merely to introduce confusion. Their paper is frequently cited in the literature by those who are apparently unaware of the true nature of the substance used. Of course it is a pure assumption, and it is an unwarranted one, to say that the results obtained by those authors are applicable to any other digitalis principle. As a matter of fact, they were attempting to explain why digalen was more active by subcutaneous injection than by vein, itself a false assumption, which they might have discovered by the simple experiment of injecting suitable doses of the drug subcutaneously and intravenously into animals. This paper is mentioned here merely as an example of those through which confusion has been introduced into the literature by careless generalizations.

Yernaux (11) found that digitoxin (there termed Nativelle's digitaline), disappears rapidly from the blood following its intravenous injection into the rabbit. He was unable to detect the poison by means of the biologic test on the frog's heart in blood taken one minute after the injection of the digitoxin. He found the chemic test for digitoxin unsuitable for the examination of blood.

Yernaux observed that digitoxin dissolved in horse serum (which is said to be a convenient vehicle) is more toxic by subcutaneous injection than an equal amount dissolved in alcohol or dilute alcohol.

Werschinin (12) found that the use of serum or blood as a vehicle for digitoxin or ouabain (there called strophanthin-g) greatly increased its toxicity over that in Ringer's solution,

presumbably because some constituent of the serum increases the rate of penetration of the poison into the heart. He also found that lecithin (13) increased the rate of penetration of the poison into the heart but did not account for the increased toxicity, hence there is some other constituent of the serum that is concerned in this action.

Schliomensun (14) prepared from the hearts of men and animals a group of substances (alcohol-soluble phosphatids), which appeared to be capable of binding digitoxin, while corresponding fractions from skeletal muscles and liver had no such binding power.

Oppenheimer (15) found that serum, used as a vehicle, increased the activity of ouabain (there called strophanthin crystalline) and antiarin, but that it distinctly interfered with the activity of digitoxin, gitalin, digitalin (?), saponin, and methyl violet during perfusion of hearts of *Rana temporaria*. He maintained that lecithin and cholesterin are not responsible for the serum effect. He held that the disappearance of digitalis bodies (as indicated by the inability to detect them) that have passed through the organism cannot be attributed to destruction or to fixation in the cells.

Hatcher (16) found that when ouabain (there called crystalline strophanthin) is injected subcutaneously into the rat the larger part of it is excreted into the intestine, and a smaller part in the urine. The total amount recovered from the urine and intestine, as shown by the biologic estimation on cats, was nearly equal in one case to the amount injected. The mode of elimination into the intestine was not determined at that time. He administered a massive dose of ouabain (50 mgm.) to a rat by the mouth and recovered about 75 per cent of it from the gastrointestinal tract about twenty-six hours later. He was unable to obtain evidence that the heart tissues of the rat fix ouabain, despite the well known fact that that animal is extremely tolerant toward many of the digitalis bodies.

Hatcher and Bailey (17) sought to determine whether the liver of the dog is capable of fixing or destroying ouabain (there called crystalline strophanthin). They found no difference

between the amount required to cause death when the poison was injected slowly into the splenic, and that required when the injection was made into the femoral, vein. They were seeking to determine whether fixation in the liver accounts for the slighter toxicity of the poison after oral administration, as compared with that after subcutaneous injection. As a matter of fact, it is probable that very little of the poison is fixed during a single passage through the liver of any animal, except the rat, among those commonly used in laboratory experiments.

Dale and Laidlaw (18) tested the urine of the cat for apocynamarin biologically after the daily administration of doses of 2 mgm. for eight days. The results of the tests of three specimens of urine were uniformly negative. They state that the question of its elimination unchanged or its destruction in the body remains open, but they regard the negative results just mentioned as favoring the view that it is destroyed in the body. Apparently they did not take into consideration the possibility of its elimination in the bile.

Focke (19) states that the digitalis bodies combine with the cells of the heart muscle and that their slow excretion from the blood is the principal cause of the cumulative action that is characteristic of this class of poisons. Focke's statement is frequently quoted, but we are not aware of any experimental evidence upon which his assertion rests.

Straub (20) states that it is impossible to recover digitalis bodies from the poisoned hearts of frogs. The hearts were successively brought to systolic standstill with a solution of ouabain (there called strophanthin) with an apparent loss of 0.0002 mgm. of ouabain for each heart so brought to standstill. He concludes that this does not constitute "storage" of the poison in the heart in the sense in which that term is commonly understood, though he admits that storage may be said to occur to the extent of 0.0002 mgm. for each heart brought to standstill in that way.

Issekutz (21) found that frogs' hearts took up 0.00075 mgm. each of digitoxin, or about three and half times as much by weight as of ouabain, as observed by Straub. He believes that the digitoxin is firmly bound in the heart.

Gruenwald (22) argues that storage does occur in the heart. He employed Merck's "pure digitalin," which consists of a mixture of true digitalin and digitonin, and A. D. Waller (23) states that, "Digitalinum pulverisatum purum Germanicum" gives a characteristic record on muscle that is quite indistinguishable from a saponin record. Obviously we are not justified in accepting the results obtained with this digitalin as being applicable to any true digitalis body. See also Straub's reply (24) to Gruenwald and an article by Weizsaecker (25).

Lhoták (26) found that an amount of digitoxin equal to ten times the fatal dose disappeared from the blood of the rabbit almost immediately after its intravenous injection. He employed the frog test in the examination of specimens of blood taken from the rabbit immediately after death, which followed promptly the intravenous injection of very large doses of digitoxin. He was able to detect the poison in the organs of the animal after the intravenous injection of amounts equal to more than ten times the minimal lethal dose. Animals in a pathological condition with impaired functions, and those narcotized with alcohol or hydrated chloral did not eliminate digitoxin from the blood so rapidly as the normal animal.

The action was slower, and larger amounts were required to cause death, when the poison was injected into the crural artery than when it was injected into the femoral vein. About 20 per cent of the amount injected into the crural artery could be detected in the muscles supplied by that artery but none could be recovered from the corresponding muscles of the opposite side. He concluded that digitoxin is certainly fixed in the heart and walls of the vessels. He found that ouabain disappeared more slowly than digitoxin from the blood.

Lhoták also employed cross circulation between two rabbits, with a brief interruption in the cross circulation immediately after the injection of digitoxin. In some cases the injection of amounts of digitoxin equal to several times the fatal dose for the first animal failed to cause death in the other. We have cited Lhoták's results and conclusions at some length because of their immediate bearing on our own investigations.

Hatcher (27) injected digitoxin into cats, dogs, and a rat, and ouabain into cats and rats, and estimated the amounts of the poison remaining in the blood at death, or after various intervals of time, by injecting the defibrinated blood into another animal of the same species except in the case of the rat, the blood of which was tested by injecting it into cats. He found that about one-fourth of the digitoxin injected intravenously into dogs was present in the blood at death after six minutes; the greater part of massive and moderately large doses of ouabain disappeared rapidly from the blood of the cat, about one-third of the total amount injected intravenously being present in the blood at the time of death, which occurred within about two minutes. Ouabain also disappears rapidly from the blood of the rat. Owing to an oversight it is stated in Hatcher's paper that digitoxin disappeared completely from the blood of a rat in one minute; it should have been in one hour and forty minutes; no attempt was made at that time to determine the rate of disappearance of the poison from the rat's blood more precisely.

Hatcher also injected ouabain into the femoral artery of the cat and observed that death was delayed beyond the time when it would have occurred had the same dose been injected into the femoral vein. He concluded that these digitalis bodies are distributed widely through the tissues of the body following intravenous injection, but a footnote was added to the paper while it was in press stating that the liver of the rat fixes ouabain promptly after its intravenous injection.

Hatcher also reported that when a dose of 100 mgm. of ouabain is administered to a rat by the mouth the greater part of it disappears during the passage of the alimentary tract, being apparently destroyed by the successive action of ferments or by bacteria, since very little appeared in the feces and no symptoms of poisoning occurred despite the enormous amount used.

Clark (28) employed Kombe strophanthin only, prepared by Burroughs, Wellcome and Company, in an investigation of the mechanism of tolerance of the rat and snake to glucosids of the digitalis group. He tested extracts of the tissues on the frog's heart but found no evidence that any of the tissues of the rat, frog,

snake, or rabbit are capable of destroying strophanthin; that the red blood corpuscles of the frog in the presence of plasma possess the power of absorbing small quantities of strophanthin, but the mammalian blood has no such power. Perfusion of the rat's heart with a solution containing 0.005 mgm. of strophanthin in each cubic centimeter of the fluid caused systolic standstill in one hour; perfusion with a solution containing a total of 0.01 mgm. caused systolic standstill in four hours.

Especial attention is called to the fact that Clark worked with Kombe strophanthin, almost certainly an authentic specimen, and it is fair to assume that he used the amorphous. Unfortunately, Clark falls into the error of generalizing, at least in so far as the title of his paper is concerned—"The Factors Determining Tolerance of Glucosides of the Digitalis Series"—but in his conclusions he is careful to refer in every case to strophanthin alone. Clark also found no evidence that the liver of the frog, rabbit, or rat has any power to absorb strophanthin during perfusion experiments. We have taken pains to investigate the capacity of the rat's liver to fix amorphous strophanthin in view of the remarkable power that it exhibits for fixing the nearly related ouabain.

Groeber (29) suggested that the actions of amorphous and crystalline strophanthins from Kombe strophanthus and the amorphous strophanthin from hispidus strophanthus might be due to strophanthidin resulting from the decomposition of the strophanthin in the organism. He based this view on the observation that the effects of strophanthidin are elicited almost immediately after its intravenous injection in the rabbit, whereas there is always an interval of some minutes following the injection of the smallest effective doses of strophanthin before the effects are induced. There is no necessary connection between these two observations, however, and Groeber fails to explain why he found strophanthin to be about three and a half times as active as strophanthidin. He states that the question is still an open one.

The greater number of the active principles of the digitalis group are glucosids and many attempts have been made to de-

termine to what extent they are decomposed by digestive ferments, acids, and alkalis under conditions simulating those found in the alimentary tract.

Deucher (30) digested true digitalin with hydrochloric acid, with hydrochloric acid and pepsin, and with pancreatin and determined the degree of decomposition by means of the frog test. He concluded that 0.2 per cent hydrochloric acid decomposes true digitalin, and that the addition of pepsin increases the rate of decomposition, but that pancreatin is without effect on the digitalin.

Krause (31) states that the injection of diastase at once after the administration of strophanthin (source not stated) was followed by recovery in a series of experiments in which amounts equal to five times the minimal lethal dose were given. He does not state the mode of administering the strophanthin, but the context suggests that it was injected subcutaneously. This observation has never been confirmed, so far as we know, but, on the contrary, we found that the intravenous injection of diastase was without perceptible effect on the course and degree of the intoxication when Merck's amorphous strophanthin (Kombe?) was injected intravenously at the same time. Krause's statement is important if correct, but we are unable to accept it.

Loewy (32) extended Deucher's observations and investigated the action of acid, alkali, and acid with pepsin on strophanthin (amorphous Kombe?), helleborein, and infusion of digitalis. Strophanthin was not decomposed; helleborein was decomposed only to a slight extent, or not at all; Loewy's statement is ambiguous, but the protocol of his experiment shows no decided action; acid decomposed the infusion of digitalis, the addition of pepsin to the acid having little effect on the rate of decomposition.

Loewy's paper is not convincing. He found that the infusion of digitalis lost nearly half of its activity when it was allowed to stand at room temperature for twenty-four hours, becoming practically inert within a period of seventy-two hours. This is entirely at variance with every day clincial experience and with our own experimental evidence. We (33) found the infusion of digitalis to be remarkably stable when kept for several weeks under a variety of conditions, and in one case (unpublished) we

found that a specimen of the infusion which had been allowed to remain in an incubator at 37°C. for nearly three years retained about half of its original activity. No one, of course, denies that acids destroy glucosids in time.

Hatcher (34) found that the digestion of ouabain (there called crystalline strophanthin) and tincture of strophanthus (Kombe or hispidus?) with pepsin and acid, and with pancreatin and alkali for two hours resulted in some loss of activity, but the apparent loss was within the limits of error of the experiment.

Worth Hale (35) employed the frog test to determine the degree to which various digitalis bodies are decomposed under conditions simulating those in the human alimentary tract. He found that hydrochloric acid decomposed digitoxin slowly, the addition of pepsin then being without influence on the rate of decomposisition, while pancreatic digestion did not affect the digitoxin; French digitalin, (said to consist largely of true digitalin) digitalein, and infusion of digitalis, behaved essentially like digitoxin, strophanthin, (probably amorphous from Kombe strophanthus) was decomposed more rapidly.

Lhoták (36) administered digitalis to rabbits by the mouth in amounts up to thirty grams and sought to determine whether it could be detected in the gastro-intestinal tract, blood, liver, heart, urine, or feces. He detected it in the stomach occasionally (which is obviously to be expected), never in the small or large intestine, organs or excreta. He concluded that the rabbit destroys digitalis in the stomach and small intestine, and that this capacity for destroying it increases with habituation. His results are not convincing that the destruction occurs in the stomach and small intestine alone, since absorption followed by destruction elsewhere may occur, and we know that rabbits are capable of disposing of digitoxin rapidly after its intravenous injection (37).

Holste (38) treated infusion of digitalis and solutions of crystalline digitaline, digitoxin, ouabain, Boehringer's strophanthin (probably the amorphous, from hispidus) and helleborein with digestive ferments, diastase, and emulsin for periods varying up to twenty-four hours. He found that the activity of the infusion is destroyed readily, that of digitoxin and strophanthin

less readily, and he concluded that much of the uncertainty following the clinical use of digitalis results from the splitting of the active principles by digestive ferments.

This paper is one of those that serve to confuse, rather than clarify this subject. It appeared with the prestige of having come from Schmiedeberg's laboratory, but it is not convincing. An examination of the results actually obtained does not afford evidence that any uncertainty in the therapeutic action of these agents is due to splitting by digestive ferments. The destruction of ouabain and digitoxin was unimportant in amount, and those agents do not usually remain for periods of twenty-four hours in the stomach or duodenum, where absorption occurs.

Johannessohn (39) found ouabain (there called crystalline strophanthin-g) much more resistant than amorphous and crystalline Kombe strophanthins to hydrochloric acid and gastric juice. He also found hydrochloric acid and pepsin together less actively destructive of strophanthin than hydrochloric acid alone in similar concentration, his explanation being that pepsin bound a portion of the acid. Johannessohn's conclusions are more in accord with known facts than those of Holste just mentioned.

Certain salient facts mentioned in the literature just presented may be reviewed here because of their relation to the results of our own experiments. Their discussion will be reserved until we have detailed these experiments, in which we have sought to determine so far as feasible, the several stages in the elimination or destruction of ouabain, amorphous strophanthin, and digitoxin in the rat, cat and dog.[3]

There has been no satisfactory evidence afforded hitherto that any of the glucosids of this group are fixed in any of the tissues or organs of the body in amounts that permit their recovery and quantitative estimation.

There is no convincing evidence that any of the digestive juices or their ferments has any important destructive action on any

[3] We regret that our inability to secure a supply of rabbits has interfered with our plan to continue our studies with this animal, begun several years ago, but its behavior toward these principles probably differs from that of the rat only in degree.

of the digitalis glucosids following their therapeutic administration by the mouth. But of course it is well known that dilute acids destroy all glucosids, including those of the digitalis group, in time.

Digitalis is by far the most important member of the group from a clinical point of view despite its well known disadvantages, but it varies in its content of the several active principles, hence we preferred to employ the active principles in the purest form available in our investigations, though this method has a disadvantage in that we do not know whether any of these principles exist in the leaf in the form in which they are isolated, and the active principles contained in the leaf may therefore behave somewhat differently. There is some evidence—not conclusive however—that the isolated digitalis principles are not identical with those existing in the leaf, but, on the other hand, there is a fairly close agreement between the actions of digitoxin and those of digitalis when they are administered orally, or intravenously in moderate doses, in which case the effects of saponin-like substances do not play an important rôle as they probably do in the almost instantaneous death that results from the intravenous injection of massive doses of extracts of digitalis.

It will be convenient to present the results of our studies under several headings, but the experiments cannot be recorded in strict chronological order because observations made in one series often necessitated the reinvestigation of problems in other series by newer methods.

II. ELIMINATION OF OUABAIN IN THE RAT

Ouabain was chosen for the first of the series of experiments because it is the only readily available pure crystalline principle of the digitalis group.[4] A further advantage of ouabain lies in the fact that it is easily soluble in water and can be extracted conveniently from animal tissues, and the amounts so recovered can be estimated quantitatively by means of the biologic test on

[4] All of our specimens of ouabain contain traces of insoluble impurity, but this is without significance in its experimental or clinical use.

the cat without the necessity of purifying the extracts beyond such simple methods as precipitating protein by heat and subsequent filtration.

The rat was selected for use in the first series of these experiments because its extraordinary tolerance toward the various digitalis bodies permits of the administration without harm of such amounts that the recovery of a fraction of that administered suffices for its quantitative estimation by means of the biologic test on cats.

The cat reacts fairly uniformly toward ouabain which is fatal in intravenous doses of 0.1 mgm. per kilogram of weight, and this holds true whether the poison be dissolved in salt solution, in defibrinated blood, in urine (if not in an excessive amount of the latter), in extracts of feces, or in extracts of tissues, but the latter must be injected slowly in most cases.

The method of extraction and estimation used with unessential modifications in the present investigation may be described briefly as follows: Blood is defibrinated and diluted, if necessary, so that it contains about one part of ouabain in 100,000 parts of solvent, it is then injected slowly and continuously into the femoral vein of the test animal until death occurs, the duration of the injection being approximately an hour or an hour and a half. Since 0.1 mgm. of ouabain is fatal to a kilogram of animal, the total amount of ouabain present in the blood can be calculated from the amount of blood required to kill the test animal. From one to three tests were made, depending upon the accuracy required in the experiment, and the amount of material available. Urine is tested in the same way as the diluted blood, being diluted with normal salt solution when there is reason to suppose that it contains more than one part of ouabain in 100,000 parts of urine. Tissues are ground with sand, or in a hashing machine, heated on a water bath with water or normal salt solution, cooled, filtered, and tested in the manner just described. Feces are collected in alcohol, ground in a mortar, the alcohol evaporated, and the residue extracted with water or normal salt solution on a water bath, cooled, filtered, and tested in the same way as the defibrinated blood.

When the total amount of ouabain present in the tissue, or other extract is insufficient to cause the death of one test animal we employ a method which we shall refer to briefly as the "combined ouabain" method of estimation. This consists in injecting all of the extract, or so much as may be deemed expedient, usually not more than 50 cc. for each kilogram of body weight, and completing the injection with a solution of ouabain of known concentration in normal saline. The difference between the amount of ouabain thus required in pure solution, and 0.1 mgm. for each kilogram of the body weight of the test animal is the amount contained in the injected extract. This "combined" method of estimating the amounts of ouabain present is also applicable to the estimation of any other digitalis body, the activity of which for the cat is known. If 10 per cent of the normal fatal dose of ouabain is required after the injection of an extract of any digitalis body it follows that the extract contained 90 per cent of the fatal dose of that body, from which the total amount present in the extract can be calculated.[5]

Absorption and destruction of ouabain in the alimentary tract of the rat

Since ouabain is excreted partly in the urine following its subcutaneous injection, its absence in amounts that could be detected in the urine of the rat following the oral administration of amounts equal to hundreds of times that which suffices for its detection in pure solution seemed to indicate that no absorption occurred from the alimentary tract, but we have sought to determine this point with certainty, and whether it is destroyed in the alimentary tract; if so where, by what means, and the approximate rate of such destruction.

Ouabain is fixed in the liver of the rat and excreted promptly into the intestine following its intravenous injection, as will be shown subsequently, therefore, it seemed possible that the failure to detect the poison in the urine following the oral administra-

[5] For a discussion of this "combined" method see the paper of Hatcher and Brody, Am. J. Pharm., lxxxii, 360, 1910.

tion of massive doses might have been due to its fixation in the liver and excretion back into the intestine. We have, therefore, examined the liver, urine, and feces of rats after tying the common bile duct and administering massive oral doses of ouabain.

Absorption of ouabain after oral administration

A white rat, weighing 210 grams, was anesthetized, and the common bile duct was tied and cut.

The animal received 80 to 90 mgm. of ouabain in suspension, orally.

The urine was collected for a period of 22.5 hours, during which no stools were passed; the animal was then chloroformed, and the liver, the gastro-intestinal tract with contents, and the kidneys with the bladder were removed and extracted; the extract of the kidneys and bladder being added to the collected urine before testing. The amounts found were as follows:

Gastro-intestinal tract and contents	50.1 mgm.
Urine, kidneys and bladder	0.7 mgm.
Liver	0.0 mgm.
Total	50.8 mgm.

The protocol of this experiment shows that less than 1 per cent of the ouabain administered orally to the rat was present in the urine, and that the liver contained none, hence less than 1 per cent of the poison appeared to have been absorbed. The intestine with its contents contained about half of that administered, and we must suppose that nearly all of that which disappeared was destroyed, and, as we shall show subsequently that the liver and tissues do not destroy large amounts of it, we must suppose that the destruction occurred in the gastro-intestinal tract.

In another experiment, of which a condensed protocol is given, the bile duct was tied, after which a dose of about 100 mgm. was administered orally, and the feces, collected for five days, contained only about 2.3 mgm. of the poison.

Protocol of experiment (condensed). White rat, weight 127 grams

August 29. Common bile duct tied during ether anesthesia. *1.35 p.m.* 125 mgm. ouabain in emulsion into stomach through blunt needle; slight loss; actual dose lay between 100 mgm. and 125 mgm.

August 31. 4.00 p.m. No feces passed; urine collected, called first portion, diluted to 50 cc.

September 3. 11.30 a.m. Urine collected, second portion, diluted to 100 cc., feces extracted, made up to 100 cc.

Summary of results of tests:

Urine, first portion, contained	0.93 mgm. ouabain
Urine, second portion; contained	0.00 mgm. ouabain
Extract of feces contained	2.33 mgm. ouabain
Total recovered	3.26 mgm. ouabain

In order to determine the seat, and approximate rate, of destruction of ouabain in the alimentary tract of the rat measured amounts were administered orally, and after varying intervals of time we determined the amount of the poison remaining in the stomach and intestine, or in certain parts of the alimentary tract, and in some cases the amounts present in the feces.

In one experiment 20 mgm. of ouabain were administered to a rat by the mouth; the animal was killed after two hours, and the entire amount of the poison administered was recovered from the stomach. A similar dose was administered orally to another rat which was chloroformed after twenty-four hours; the gastro-intestinal tract and feces together contained 13 mgm. indicating the destruction of about one-third of the total amount administered, absorption being negligible. Another rat ate about 40 mgm. of ouabain, which had been mixed with cracker dust, during a period of about two hours. The animal was killed four hours after having eaten the poison, at which time, the stomach contained none, the small intestine contained 1 mgm., and the large intestine contained 20 mgm., the results indicating the destruction of about half of that administered. Four milligrams of ouabain were recovered from the gastro-intestinal tract of a rat twenty-four hours after the oral administration of 10 mgm.

These experiments show that ouabain is destroyed at a variable rate, but on the whole fairly rapidly, in the rat's alimentary tract and we undertook to determine the immediate seat of this destruction.

Four rats, weighing 125, 133, 145, and 195 grams respectively, were given equal amounts of ouabain, the dose being 10 mgm. in

every case. The smallest rat received the poison directly into the stomach through a blunt hypodermic needle which was passed down the esophagus; this method prevents troublesome regurgitation such as occurs frequently when the poison is placed in the mouth or throat. The second rat was anesthetized and the ouabain injected directly into the duodenum in order to avoid the action of the gastric juice; the third was anesthetized and the poison injected directly into the ileum immediately above the ileo-cecal valve with the intention of having it pass into the large intestine without its traversing the stomach or small intestine. The fourth animal received the ouabain by subcutanaeous injection.

The feces of all of these animals were collected separately in alcohol for a period of four days, extracted, and tested in the usual manner. The animal that received the poison directly into the stomach excreted 2.5 mgm. in the feces; that which received it directly into the ileum excreted 0.8 mgm., and the other two did not excrete any. It is not a coincidence that the least destruction occurred in the animal in which the poison traversed the entire gastro-intestinal tract, while all, or very nearly all, of the poison was destroyed in those cases where it traversed only a part of it. The animal that received the ouabain directly into the stomach suffered no ill effects whatsoever, peristalsis continued actively and feces were passed abundantly, hence the poison remained for a much shorter time in the intestinal canal in this case than in the others; two of which suffered severe depression from the anesthesia and the operation of opening the abdomen, and one from the relatively large dose of ouabain administered subcutaneously. Strictly analogous results followed in a similar series of experiments with the closely related amorphous strophanthin.

The results of the experiments just detailed point to the large intestine of the rat as the seat of active destruction of ouabain, and they suggest strongly that it may be the only part of the gastro-intestinal tract in which destruction of the poison takes place actively. We have tried to determine approximately the rate of this destruction by injecting weighed amounts of ouabain directly into the cecum, or mixing them with the contents and ground intestinal tissue, allowing them to remain in an incubator

at 38°C. for some hours, and determining the amount of ouabain remaining undestroyed, by means of the biologic test on the cat. The results of the experiments were far from uniform, and in some cases little destruction, if any, occurred; in others the destruction was less than we anticipated in the light of the results with the living animal.

We have no data upon which to base a positive statement by way of explanation of the discrepancy, but it is obvious that the processes that go on in the intestine of the living rat are quite different from those that take place in an excised and incubated intestine.

It is hardly necessary to say that we had previously convinced ourselves by experimental evidence that extracts of intestinal contents could be injected intravenously in the cat without inducing perceptible effects, and without affecting the course of poisoning by ouabain. The injection of an excessive amount of extract of the contents of the dog's large intestine caused depression with loss of appetite lasting a week or more in one case. The extracts of the intestinal contents contain some substance having a feeble hypnotic action, but this is not observed with the relatively small amount obtained from the rat (if it exists in that animal). It may be mentioned here in passing, that extracts of the rat's liver sometimes induce emesis in the cat when administered orally, and the uncooked liver of the cat appears to induce emesis, when eaten by another cat, more actively than an equal amount of raw meat.

It is evident that the extraordinary tolerance of the rat toward ouabain administered orally rests primarily on its want of absorption, for destruction does not then take place until the large intestine is reached. It is an interesting fact also that this animal resists almost completely the absorption of ouabain, which is readily soluble in water (as well as that of amorphous strophanthin, which is even more soluble), while it absorbs readily the practically insoluble digitoxin, after their oral administration. It is of further interest to observe that the cat, dog, rabbit, and even man, do not differ fundamentally from the rat in this respect, but only in degree, enormous oral doses of ouabain having been survived by man.

The elimination of ouabain after subcutaneous injection in the rat

This has been the subject of a paper by one of us (Hatcher) and it requires only a brief discussion here.

The rat is nearly one thousand times as resistant to ouabain as the cat, in proportion to its weight, when the poison is injected subcutaneously. The cause of this tolerance is evidently different from that seen after oral administration, for absorption from the subcutaneous tissues occurs fairly promptly, and an animal may die within a short time after the subcutaneous injection of a fatal dose.

The fatal intravenous dose of ouabain for the cat is 0.1 mgm. per kilogram of weight, regardless of the rate of injection within wide limits, but the minimal fatal dose for the rat depends largely on the rate at which the poison enters the circulation. The rapid intravenous injection of as little as 12 mgm. per kilogram of weight may be fatal almost immediately, but an amount roughly equal to 100 mgm. per kilogram may be injected subcutaneously without causing death, although practically all of this huge dose enters the circulation and is in turn excreted within a few hours.[6]

The protocol in brief of an experiment serves to show the approximate rate of absorption of ouabain from the subcutaneous tissues of the rat and its elimination into the intestine.

Protocol of experiment

A rat, weighing 202 grams, received 10 mgm. of ouabain subcutaneously; the animal was chloroformed after one hour and fifteen minutes, and the liver and the small intestine were removed and extracted separately, and the extracts tested in the usual manner.

The extract of the liver contained 2 mgm. of ouabain, that of the small intestine 4.24 mgm.

The result of this experiment shows that 6.24 mgm., or more than twice the fatal intravenous dose, had passed through the circulation in a period of one hour and fifteen minutes without causing death.

[6] The average fatal dose of ouabain for the rat by intravenous injection is about 10 to 15 mgm. per kilogram of weight when a solution of one part in two hundred of normal saline is injected within a few seconds, but death is usually somewhat delayed after the smaller dose.

One may suppose that this is approximately the maximum rate of elimination of ouabain of which the organism is capable, and this view receives support from the results of repeated subcutaneous injections of large doses at intervals of two hours. In one such experiment a total amount equal to fifteen times the single fatal vein dose was administered to each of two rats within a period of four hours. One of the animals survived, the other died one hour and fifteen minutes after the last dose was injected. As a matter of fact we shall have occasion to show that the liver of the rat is sometimes capable of removing ouabain from the blood even more rapidly than these experiments indicate, but the rate at which an organ can fix and remove a poison from the blood is not always an index of the rate of elimination of the poison from those organs upon which its essential, or toxic, action is exerted. We are inclined to speak of this latter as the "essential" rate of elimination, in order to distinguish it from that which may take place immediately after the poison enters the circulation. It is evident that we are dealing with an important factor in persistence of action, but this phase of the question cannot be discussed here; it may be said, however, that animals do not recover completely even as rapidly as the rate of elimination after subcutaneous injection would lead one to suppose, and even where recovery does eventually follow such large and repeated subcutaneous doses, there are symptoms referable to the central nervous system which persist for many hours after the administration of the last dose. It is more than possible that these symptoms are caused by the fixation of minute portions of the poison and their retention in the central nervous system.

The protocol of an experiment showing the effects of repeated doses of ouabain injected subcutaneously follows:

Protocol. White rat, weight 40 grams

10.00 a.m. 60 mgm. ouabain per kilogram subcutaneously in N. S. 1–200.

10.10 a.m. Sick, tremulous, prostrated.

12.00 a.m. Depressed but recovering; 60 mgm. ouabain per kilogram as before; symptoms much like those after preceding dose.

2.00 p.m. 60 mgm. ouabain per kilogram as previously. The animal had partly recovered by the following morning, but was tremulous and hyperexcitable. Complete recovery finally took place.

We have frequently confirmed experimentally the observation of Hatcher (16) that approximately 15 per cent of the ouabain injected subcutaneously into the rat may be excreted in the urine, but the rate of elimination in this way is quite variable, and at times almost negligible.

It is significant that in some experiments Hatcher recovered from the urine and intestine of the rat nearly all of the ouabain that had been injected subcutaneously when toxic, but not fatal, doses had been used, whereas in several experiments we failed to recover amounts comparable to those injected. It is unnecessary to give the protocols of these, but in the light of our experiments that show that destruction takes place in the large intestine of the rat, but apparently not in the small, it seems probable that differences in the amounts destroyed account for our want of concordant results. Intestinal peristalsis in the rodent depends to a remarkable degree upon the food taken, hence it seems probable that in some cases the poison excreted into the small intestine remained there for a considerable period of time, and thus escaped destruction, or that it was recovered from the large intestine before destruction had progressed far, whereas in other instances the delay occurred in the large intestine permitting of more being destroyed.

Several experiments of this series were performed as class experiments, the tests being conducted by students under our supervision, and the data are not complete in every case, nor are the intervals recorded with precision in all of those, but we will tabulate the results obtained so far as we can. It is of especial interest that in one experiment the liver was found to contain as much as 2 mgm. of ouabain after the subcutaneous injection of 10 mgm. because we shall have occasion to show that in some cases the poison is passed on into the intestine from the liver very rapidly after the intravenous injection of much smaller doses. This affords convincing evidence that the liver may store the poison

when it passes gradually into the circulation, as it does after its subcutaneous injection.

Table 1 shows the dose of ouabain in milligrams; the interval in hours (or approximately) after the injection and before the

TABLE 1

Showing the excretion of ouabain in the rat

WEIGHT	DOSE	RECOVERED IN		DESTROYED		INTERVAL
		Urine	Feces			
After oral administration						
grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*hours*
	50.0		30.0	20.0	40	22
	50.0		37.5	12.5	25	26
110	10.0		3.9	6.1	61	24
	20.0		13.6	6.4	32	24
150	40.0		21.0	19.0	48	4–6
125	10.0		2.7	7.3	73	96
165	100.0		25.0	75.0	75	48
		(bile duct tied)				
127	100–125	0.93				50
			2.33		97	118
210	80–90	0.70	50.1		40	22½
After intestinal administration						
145	10.0		0.9	9.1	91	96
135	10.0		0.0	10.0	100	96
143	10.0		8.5	1.5	15	22*
91	10.0		1.4	8.6	86	22*
After subcutaneous injection						
	10.0	0.85	4.1	5.1	51	18
	15.0	3.00	10.0	2.0	13	24
195	10.0		0.0			96
	10.0	0.16	2.8	7.0	70	24
270	15.0		4.6			24
297	5.0	0.60	1.1	3.3	66	18
180	10.0	1.80	2.9	5.3	53	18
200	20.0	3.00	4.8	12.2	61	48
85	8.5	0.50	2.5	5.5	65	24
110	10.0	1.50	4.9	3.6	36	24
150	10.0					96
150	10.0	12.0†		12.0	60	96

TABLE 1—*Continued*

WEIGHT	DOSE	RECOVERED IN		DESTROYED		INTERVAL
		Urine	Feces			
After intravenous injection						
grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*hours*
200	2.4	0.00	1.25	1.16	48	42
210	2.0	0.16	0.43	1.4	70	44
		(bile duct tied)				
	2.0‡	2.12	0	0	0	20
230	2.0	1.15				44

* The intestine was tied above the cecum in both of these; the injection was made into the duodenum of the first, and into the cecum of the second.

† This amount was recovered from the urine and feces together of the two rats, each of which received a dose of 10 mgm.

‡ The bile duct was tied and this dose was administered ninety-six hours after a similar dose.

excreta were examined: the amounts found in the urine, and in the intestinal tract with its contents and any feces that were excreted during that period. In one experiment the intestinal tract was extracted after removing its contents but none of the poison was recovered from its tissues. In one experiment a rat, weighing 210 grams, excreted 0.17 mgm. of ouabain in its urine in forty-four hours after the intravenous injection of 2 mgm. The bile duct was then tied and the animal excreted 2.12 mgm. in the urine in twenty hours after a similar dose; this may possibly include a small residuum from the previous dose. Renal excretion after oral administration in the normal animal was disregarded in estimating the amounts destroyed.

The elimination of ouabain after intravenous injection in the rat

Ouabain leaves the blood stream rapidly after its intravenous injection, as previously stated, and we have undertaken to learn the mechanism of its removal and the paths that it pursues in the course of its elimination. We had previously found that the liver is concerned in the excretion of this poison, and earlier work had shown that a small part is excreted by the kidneys, after subcutaneous injection. We have endeavored, therefore, to deter-

mine whether all of the ouabain administered (excepting such minute amounts as must be taken up by the heart and central nervous system) is fixed at once by these organs.

Since nearly all of a subcutaneous dose of ouabain can be recovered from the urine and intestine of the rat within a few hours after its administration, and since its detection in the liver and blood of that animal presents no difficulty, we anticipated little trouble in recovering the poison from any of the tissues in which it might be fixed in notable amounts after the intravenous injection of doses that must be considered very large, in proportion to the fatal dose for the test animal, and in estimating the amounts so fixed. We were greatly surprised, however, to find that in some cases we were unable to recover nearly so much as we had injected when the tests were made shortly after the administration, whereas in other experiments larger relative amounts were recovered when the animals survived for longer periods.

This lack of concordant results caused us to repeat some of our experiments in order to determine whether the method of procedure was at fault, and to vary them for the purpose of attacking the problem in different ways. We will tabulate the results of the experiments of this series and discuss them in some detail. The table may give the impression that the experiments were haphazard, but most of them were performed for the purpose of solving definite minor problems, and space does not permit of our giving all of the protocols in full, but we shall give those which seem to us necessary for a clear understanding of the methods, as well as of the possibilities and limitations of these methods.[7]

[7] Chemistry has so long been in the ascendancy that many refuse to accept the view that biologic tests may be preferable to the chemical in some cases. The following quotation serves to suggest the difficulties encountered in investigations of this character: "At present nothing definite is known regarding the fate of digitalis glucosids in the human organism, or the products into which they are changed or the forms in which they are eliminated. . . . . Thus far it has not been possible to find any of the digitalis compounds mentioned above (digitonin, digitoxin, true digitalin) in blood or animal organs." (The Detection of Poisons, Autenrieth-Warren, 1915, p. 201.

Table 2 shows the dose of ouabain administered in milligrams; the duration of the injection and the interval, in minutes, following the injection before the death of the animal; the weight

TABLE 2

Showing the recovery of ouabain from the blood, liver, and intestine of the rat after intravenous injection

NUMBER	DURATION OF		WEIGHT	BLOOD		DOSE	OUABAIN RECOVERED IN		
	Injection	Interval		Total	Tested		Blood	Liver	Intestine
	min.	*min.*	*gm.*	*cc.*	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	15	5.0	156	9.8	4.5	2.0	0.25	0.49	
2	15	4.0	203	12.7	5.0	3.0	0.28	0.93	0.53
3	17	6.0	161	10.0	8.0	3.5	0.05	1.35	
4	10	2.0	73	4.5	2.25	1.5	0.06	0.52	
5	18	12.0	140	8.8	3.0	3.0	0.00	0.87	1.26
6	0	4.0	139	8.7	3.0	1.5	0.32		
Liver and small intestine extracted together in the following									
7	9	5.0	166	10.4	5.0	2.0	0.05	1.13	
8	1	6.0	280	17.5	6.8	2.8	0.00	0.60	
9	1	6.0	185	11.6		1.8		1.10	
Perfused fluid and blood taken together in the following									
10	8	1.5	202	12.7		3.0	1.33	0.80	
11	9	10.0	203	12.7		2.6	0.35	0.56	
The common bile duct had been tied in the following									
12	9	31.0	123			2.0		1.04	
13	13	11.0	134			2.5		2.18	
14	10	11.0	230			2.0		0.35	
15	11	4.0	141			2.0		0.64	
16	16	154.0	150	9.2	9.2	3.0	0.00	0.97	
17	0	5.0	208	13.0	2.5	1.5	0.22		
The liver had been removed before injection in the following experiment									
18	0	1.25	170	8.0	3.0	0.5	0.47		

of the animal; the amount of blood used for estimating the ouabain remaining in the circulation; the estimated amount of blood in the body (calculated as one-sixteenth of the body weight in grams, and expressed in cubic centimeters of blood, the mean of

the figures given by Chisholm, taken from Donaldson's book on the rat); the calculated amount of ouabain remaining in the total blood of the body; the amount of ouabain recovered from the liver; and that from the intestine.

Discussion of experiments

Reference to table 2 shows the rapidity with which ouabain leaves the circulation and we may say with certainty that practically all of a non-fatal dose disappears from the blood within six minutes after its intravenous injection in the rat.

The condensed protocol of experiment 1 will serve to illustrate the method used in estimating the rate of disappearance of the poison from the blood; one of the tests involves the employment of the "combined ouabain" method to which reference has been made.

Protocol of experiment 1. White rat, weight 156 grams

9.50 a.m. 0.5 mgm. ouabain in normal saline 1–200 intravenously every five minutes; 4 doses in fifteen minutes.

10.10 a.m. Exsanguinated by incising heart, 4.5 cc. blood, blood defibrinated, diluted to 50 cc. with N.S. Liver, 5.2 grams, extracted to make 71 cc.

Tests

Diluted blood. Cat, weight 3.25 kgm.

10.53–12.20 p.m. Injected all of diluted rat's blood intravenously.

12.25–12.45 p.m. Injected 0.213 mgm. ouabain total; death.

Calculation.

Fatal dose of ouabain for cat of 3.25 kgm. is 0.325 mgm.
Ouabain injected in pure solution 0.213 mgm.
Difference due to ouabain in blood, 4.5 cc., is 0.112 mgm.
Total amount in blood of rat's body, 9.8 cc., is 0.25 mgm.

Extract of Liver; cat, weight, 2.7 kgm.

1.05–2.34 p.m. Injected 39 cc. extract of liver; convulsions, death.

Cat, weight 2.0 kgm.

3.04–3.59 p.m. Injected 28.5 cc. extract of liver; convulsions, death.

Calculation.

39.0 ÷ 2.7 is 14.4 cc. × kgm.

28.5 ÷ 2.0 is 14.2 cc. × kgm., average 14.3 cc. × kgm.

Since 14.3 cc. equals 0.1 mgm. ouabain, 71 cc. equals 0.49 mgm.

In experiment 5 the liver and intestine contained more than 2 mgm. of ouabain at death, which occurred twelve minutes after completing the injection of a total of 3 mgm., and thirty minutes after beginning the injection. Only a small amount was recovered from the remainder of the tissues, and it is probable that this was present in the kidneys or urine. Not a trace of the poison could be detected in the blood; all of the blood that we could obtain by exsanguinating the rat was injected into a cat weighing 2.5 kgm., after which it required the full average fatal dose of ouabain.

In order to determine whether the intestine is capable of fixing ouabain and removing it from the blood, the following experiment was performed:

Protocol of experiment 13. White rat, weight, 134 grams

9.56 a.m. Common bile duct tied during ether anesthesia.

10.13–10.26 a.m. Injected 2.5 mgm. ouabain into jugular vein.

10.37 a.m. Chloroformed; liver, stomach, and intestine removed.

The liver was extracted with enough normal saline to make 100 cc.; the stomach and small intestine with enough to make 50 cc.; the remaining tissues, excepting the skin, feet, and tail, with enough to make 150 cc.

The several extracts were tested as follows: Three cats were used for testing the extract of the liver; one cat took the entire filtered extract of the stomach and intestine after which it required the full average fatal dose of ouabain to cause death; one cat took all of the available extract of the remaining tissues and a little more than half of the average fatal dose of ouabain to cause death.

The results of the tests may be summarized as follows:

The extract of the liver contained	2.18 mgm. ouabain
The extract of the stomach and intestine	0.00 mgm. ouabain
The extract of the remaining tissues	0.24 mgm. ouabain
Total recovered	2.42 mgm. ouabain

It is clear that the intestinal epithelium is incapable of excreting ouabain, except possibly in traces, and that the poison which is found in the lumen of the intestine after subcutaneous or intravenous administration is first fixed in the liver and then excreted in the bile.

We recovered 97 per cent of the total amount of ouabain injected in this experiment, more than 80 per cent being present in the liver, although the animal lived only eleven minutes after the completion of the injection and only twenty-four minutes after its beginning. The average fatal intravenous dose of ouabain injected at once is 1.5 mgm. for a rat of this size. The fact that this rat took an additional milligram in thirteen minutes indicates the rapidity with which the liver removes the poison from the blood. The result agrees fairly well with those observed in experiments in which repeated subcutaneous injections were made to determine the rate of elimination.

A glance at the tabulated results shows that the amount of ouabain found in the liver and intestine is much less in nearly every case than that which was injected, though we have every reason for believing that all of it had left the blood at the time the examination of these tissues was made. The question arises, therefore, whether this failure to recover a larger proportion of the amount administered was due to faulty methods of extraction, or to the fact that the poison was fixed, at least temporarily, in other tissues, which later give it up slowly to the blood to be fixed in turn by the liver and kidneys and gradually excreted by them.

We have been unable to answer this question with certainty but we believe that the evidence points to the fixation of the poison in the liver, and in the kidneys to a less extent, and in these organs alone, except for the negligible amounts which are fixed in the heart and central nervous system.

We know that much of the ouabain is fixed in the vessels of these organs in loose combination at first, and that it can be removed from them by simple perfusion with normal saline; we believe that it then passes into a more stable combination in the cells of the liver, and probably in those of the kidney. We know

that a part is present in the liver in such a condition that it is not removed by simple perfusion, but can be extracted by maceration of the ground liver tissue with water, and we believe that this portion has passed into the bile ducts; we were inclined at first to believe also that that portion which is firmly bound in the liver cells was not extracted by us.

We have no proof that the ouabain is at any time present in the liver in such a form that we are unable to extract it, or that that which we do extract after the removal of a part by perfusion is present in the bile ducts, but the evidence is conclusive regarding the removal of a part of it by simple perfusion, and of the presence in the liver of another part that resists removal by perfusion but is extracted easily; and certainly the rapidity with which larger percentages of the amount administered pass through the liver into the intestine point strongly to the fact that the liver takes nearly all of the poison out of the blood.

We have made numerous efforts to settle this question definitely, but without complete success. It seemed to us that if any of the ouabain were fixed in the liver cells in such a state that we failed to extract it, a longer period of extraction with fresh portions of solvent, including water and alcohol, might remove some of this residual portion, or that digestion of the liver tissues with pancreatic extract in alkaline solution might free it and permit of its estimation. We have been unable, however, to extract a trace of the ouabain from the liver residue with alcohol, or fresh portions of normal saline after extraction by the usual method, and digestion of the liver has equally failed to show the presence of more than that extracted in the usual manner.

In one of these experiments the bile duct of a rat weighing 177 grams was tied (in order to prevent loss of ouabain into the intestine) and 1.8 mgm. of ouabain were injected intravenously during a period of one minute: after six minutes the animal was exsanguinated, the liver, weighing 7.0 grams, was ground with sand and divided into two equal portions: one portion was extracted and tested in the usual manner; the other portion was digested with 25 mgm. of tested pancreatic extract with the addition of a

little sodium bicarbonate for nineteen hours, after which it was extracted and tested. The two tests of the extract of the digested portion by the "combined ouabain" method gave closely concordant results, showing the presence of 0.5 mgm. of ouabain in that portion of the liver, corresponding to the presence in the entire liver of a little more than half of the amount injected. The tests of the extract of the undigested portion were not quite so concordant, one of them indicating the presence of a little more than 0.5 mgm. of ouabain, the average for the two indicating about 0.4 mgm., corresponding to 0.8 mgm. in the entire liver. No significance can be attached to the difference in the amounts shown by the two extractions, especially, since neither indicated more than we have commonly found by the usual method of extraction.

In another experiment intended to duplicate this the test of the extract of the undigested portion indicated the presence of more ouabain than was shown by that of the digested portion. This seems to afford evidence that the liver does not hold any ouabain after extraction by the usual method.

It will be recalled that Lhoták (36) believes that digitoxin is fixed in the walls of the vessels and in the heart of the rabbit immediately after the intravenous injection of massive doses. We give the protocols of two experiments showing the behavior of ouabain immediately after the intravenous injection of a just fatal, or non-fatal, dose.

In the first of the two experiments about to be detailed (no. 10, of table 2) the blood and perfused fluid contained far more ouabain than was found in the blood alone in any of the other experiments, the amount shown being approximately equal to half of that injected. It is true that the interval following the completion of the injection was shorter than in any of the others, but little less than in experiment 4, in which practically all of the poison had left the blood. The duration of the injection—eight minutes—certainly sufficed for the removal of much the larger part of the poison from the blood, as may be seen by comparison with the tabulated results of experiments 6, 8, 9, and 17 in each of which the period between the beginning of the injec-

tion and the death of the animal was less than in the experiment under consideration.

The injection of the blood and perfused fluid in the test animals in experiment 10 was made somewhat too rapidly, and the two tests show an unusual difference, one indicating the presence of 1.13 mgm., the other 1.54, but whether we take the higher, the lower, or the average, as we have done, the amount of ouabain shown is far greater than that found in the blood in any of the other experiments. As just stated, the injection into the test animals was made somewhat too rapidly, and the effect of this is to give results correspondingly low, but the error is negligible in any event.

The discrepancy shown in the results of the two tests of the fluid perfused through the body in experiment 11 is of a different character, and such discrepancies are occasionally unavoidable. The total amount of the poison present in this fluid did not suffice for a single complete experiment without resorting to the 'combined' method, and since we had decided to make two tests of each of the fluids in this experiment the amount of perfused fluid available for each of the tests contained only a small part of the ouabain necessary to cause death, hence a correspondingly larger amount of the pure solution of ouabain was used, and a slight difference in the susceptibility of the two animals resulted in multiplying the error. There is no reason to doubt, however, that the fluid perfused through the lower part of the body contained a small amount of ouabain. It is barely possible that this was derived from the blood, since a comparison of the color of the perfused fluid with that of a dilution of a known amount of blood seemed to show the presence of more than 1 cc. of blood in the fluid. It seems more probable that the greater portion of this small amount of ouabain, if not all of it, was derived from the kidneys by perfusion of their vessels. The injection of 20 cc. of normal saline into the thoracic aorta had served to remove the greater part of the blood from the vessels of the liver and gastro-intestinal tract, hence the ouabain found in the fluid subsequently perfused through the liver could not possibly have been present in the very small amount of blood mixed with this

perfused fluid from the liver. We estimated that this perfused fluid contained 0.5 cc. of blood, or about 4 per cent of the entire amount in the rat's body.

Protocol of experiment 10 (condensed). White rat, weight 202 grams

9.52–10.00 a.m. 3 mgm. ouabain intravenously.
10.01–30 a.m. Heart stopped.

A cannula was introduced into the arch of the aorta, and normal saline perfused through the vessels, 91 cc. of bloody fluid being recovered; this was added to the blood, the mixture defibrinated, strained, and the whole made up to 150 cc.

The liver, weighing 11.5 grams, was excised and extracted, the extract measuring 61 cc.

The kidneys were extracted and the extract made up to 27 cc.

The brain and cord were extracted and the extract made up to 50 cc.

Tests

Blood (with perfused fluid).
1. Female cat, weight 3.04 kgm.
10.37–11.58 a.m. 9.7 cc. per kgm. injected intravenously; death.
2. Male cat, weight 3.25 kgm.
10.46 a.m.–12.23 p.m. 13.2 cc. per kilogram injected intravenously; death.

Calculation. First test — 9.7 cc. indicate 0.1 mgm. ouabain.
Second test—13.2 cc. indicate 0.1 mgm. ouabain.
If 9.7 cc. contain 0.1 mgm. then 150 cc. would contain 1.54 mgm.
If 13.2 cc. contain 0.1 mgm. then 150 cc. would contain 1.13 mgm.
Average 1.33 mgm.

Extract of liver:
Cat, weight 3.48 kgm.
1.15–2.37 p.m. 7.47 cc. extract per kilogram intravenously; death.
Calculation. 7.47 cc. indicate 0.1 mgm. ouabain; 61 cc. = 0.8 mgm.

Extract of kidney.
Cat, weight 2.16 kgm.
1.34–1.50 p.m. 7.4 cc. extract per kilogram.
1.50–2.11 p.m. 0.08 mgm. ouabain per kilogram; death.

Calculation.

7.4 cc. indicate (0.1–0.08) 0.02 mgm. ouabain.
27.0 cc. contain 0.07 mgm. ouabain.

Extract of brain and cord:
Cat, weight 3.18 kgm.
1.25–2.15 p.m. Injected all of the extract intravenously.
2.18–3.00 p.m. Injected 0.102 mgm. ouabain per kgm.; death.

Summary.

Blood and fluid	1.33 mgm. ouabain.
Extract of liver	0.80 mgm. ouabain.
Extract of kidneys	0.07 mgm. ouabain.
Extract of brain and cord	0.00 mgm. ouabain.
Total	2.20 mgm. ouabain.

Protocol of experiment 11 (condensed). White rat, weight 203 grams

10.50–11.00 a.m. 2.6 mgm. ouabain intravenously; death at once.

Exsanguinated, obtained 5 cc. of blood, 20 cc. normal saline injected into thoracic aorta, the fluid collected and added to blood, which was defibrinated, strained and made up to 100 cc.

A cannula was placed in the portal vein and the liver perfused with normal saline, 100 cc. of fluid being obtained; this was of a bloody appearance and a comparison of the color with that of a dilution of a known quantity of blood indicated the presence of about 0.5 cc. of blood.

A cannula was introduced into the thoracic aorta and the lower part of the body perfused, 100 cc. of fluid being obtained, in which it was estimated there was more than 1 cc. of blood.

The liver was extracted in the usual manner, the extract measuring 200 cc.

Tests

Blood. 1. Cat, weight 2.32 kgm.
12.00–2.26 p.m. 23 cc. × kgm. injected.
2.26–2.34 p.m. 0.016 ouabain × kgm. injected; death.
2. Cat, weight 3.64 kgm.
12.14–2.27 p.m. 10.6 cc. × kgm. injected.
2.27–3.52 p.m. 0.064 mgm. ouabain × kgm.; death.
Perfused fluid of liver:
1. Cat weight 2.38 kgm.
12.29–2.09 p.m. 17.9 cc. perfused fluid × kgm. injected.
2.30–3.40 p.m. 0.08 mgm. ouabain × kgm. injected; death.

2. Cat, weight 2.58 kgm.
12.38–3.00 p.m. 21.5 cc. fluid × kgm. injected.
2.30–3.59 p.m. 0.075 mgm. ouabain × kgm. injected; death.
Perfused fluid from body.
1. Female cat, weight 2.64 kgm.
1.45–2.45 p.m. 18.9 cc. fluid injected.
2.45–3.31 p.m. 0.062 mgm. ouabain × kgm.; death.
2. Male cat, weight 2.96 kgm.
1.50–2.49 p.m. 15.9 cc. fluid × kgm. injected.
2.56–4.01 p.m. 0.08 mgm. ouabain × kgm. injected; death.
Extract of liver.
1. Cat, weight 1.98 kgm.
2.32–4.16 p.m. 26.6 cc. × kgm. injected.
4.19–4.27 p.m. 0.028 mgm. ouabain × kgm. injected; death.
2. Cat, weight 3.28 kgm.
2.33–4.05 p.m. 12.9 cc. injected.
4.14–4.51 p.m. 0.077 mgm. ouabain × kgm. injected; death.

Summary.

100 cc. blood and perfused fluid (average)	0.35 mgm. ouabain
100 cc. perfused fluid of liver (average)	0.11 mgm. ouabain
100 cc. perfused fluid of body (average)	0.16 mgm. ouabain
200 cc. extract of liver (average)	0.45 mgm. ouabain
Total	1.07 mgm. ouabain.

The condensed protocols of experiments 3 and 4 are also given as they afford further evidence that ouabain is at first loosely bound in certain of the vessels and that it is not merely present dissolved in the blood. It is not feasible to make as many experiments as we would wish with a view to determining whether ouabain is present in various tissues, but since we know that the kidneys fix amounts of the poison varying up to about 20 per cent of that injected subcutaneously, we may be certain that after its intravenous injection at least a portion of those small amounts which are recovered from the body exclusive of the liver and intestine come from the kidneys. At any rate, we have failed to find evidences of the presence of more than traces of ouabain in the voluntary muscles of the rat, even though they still con-

tained some blood, following the intravenous injection of a dose of 3 mgm. in experiment 2; and the amount found in the tissues remaining after the removal of the liver, intestine, skin, feet and tail, was no greater than one might expect to find in the kidneys alone.

It is difficult to explain why the vessels of the liver bind ouabain loosely while the vessels of other regions do not, but we must suppose that if the vessels of other regions were capable of binding it loosely, perfusion of those vessels, which in their total extent far exceed those of the liver, would result in the removal of notable amounts of the poison. We have not succeeded in removing more than small amounts in this way, however, and must suppose that other vessels than those in the liver and kidneys do not have the capacity for binding more than traces of the poison. This capacity for binding the poison must be related to the excretory function of these organs. Even though the poison had passed into a second stage in the vessels of other regions whereby perfusion failed to remove it, we should expect to recover it from the muscles and other tissues by extraction, were it fixed in their vessels (cf. experiments on cats).

It is probable that small amounts of ouabain are momentarily bound loosely in the vessels of other regions and are again rapidly taken up by the blood as the liver reduces its concentration of the poison, and thus after a few minutes only traces remain in the blood and vessels of other regions except the kidney. The distribution of the poison between the blood and vessel walls seems analogous to that which we have observed during perfusion of strychnin through the liver of the dog (40). In those experiments we showed that the liver (or its vessels) bound the strychnin loosely and that it could be removed by subsequent perfusion with fresh portions of Locke's solution.

We know of no method of further investigating this phase of the problem, but the explanation offered seems probable in the light of subsequent experiments on the cat, for we believe that the behavior of ouabain differs quantitatively, rather than qualitatively, in these two species, and we have been forced to the conclusion that the vessels of various regions fix ouabain in the cat after its intravenous injection.

Protocol of experiment 3 (condensed). White rat, weight, 161 grams

9.44–10.01 a.m. 3.5 mgm. ouabain injected intravenously; animal was killed and exsanguinated at once.

5 cc. of blood were collected, after which 5 cc. of normal saline were perfused through the aorta, the escaping fluid collected and added to the blood which was defibrinated, diluted to 50 cc., and tested.

The perfusion of the vessels through the aorta was continued with repeated passage of the first portion, and the perfused fluid finally made up to 290 cc. A comparison of a dilution of the blood with the perfused fluid indicated the presence of about one-fourth as much blood in the perfused fluid as in the 5 cc. of diluted blood.

The liver, which was practically bloodless, was extracted in the usual manner, and the extract made up to 50 cc.

One test was made of the blood; two of the perfused fluid, which showed close agreement; and two of the extract of the liver, which were in exact agreement.

The results of the test show:

Blood (estimated at 80 per cent of the whole)	0.042 mgm. ouabain
Perfused fluid	0.137 mgm. ouabain
Extract of liver	1.35 mgm. ouabain
Total amount recovered	1.529 mgm. ouabain

Protocol of experiment 4 (condensed). White rat, weight, 73 grams

9.50–10.00 a.m. 1.5 mgm. ouabain injected intravenously.

10.02 a.m. Death; exsanguinated.

Blood, measuring 2.25 cc. (estimated one-half of whole) diluted to 25 cc.

Vessels perfused with normal saline to make 97 cc.

Liver, nearly bloodless, extracted, extract made up to 50 cc.

Skin, feet, and tail removed and the remaining tissues, weighing 59 grams, extracted, the extract made up to 200 cc.

The results of the tests (all by the "combined ouabain" method) show:

Blood (1 test)	0.06 mgm. ouabain.
Perfused fluid (2 tests)	0.52 mgm. ouabain.
Extract of liver (1 test)	0.50 mgm. ouabain.
Extract of remaining tissues (including kidneys) (1 test)	0.30 mgm. ouabain.
Total amount recovered	1.38 mgm. ouabain.

In the effort to learn definitely whether the liver plays such a preponderant rôle in the immediate removal of ouabain from the blood of the rat (its ultimate rôle in eliminating the poison not being just now under consideration), and whether other organs are capable of fixing it temporarily, at least, we performed three experiments which seem to us to throw some light on the question.

The first of these experiments was made on a rat weighing 170 grams; the liver was removed, a dose of ouabain equal to one-fourth of the average fatal dose for an animal of this size was injected intravenously, and the blood tested after death, which occurred after sixty-five seconds. The protocol of this experiment shows that little of the ouabain, if any, had been removed from the blood.

We are well aware that numerous objections may be urged against the results observed in this experiment. The removal of the liver of an animal can hardly fail to influence the behavior of almost any poison after its intravenous injection even though it were not one of those that the liver fixes actively, for such a profound change in the circulation as this operation involves could hardly fail to have some effect, to speak of no other possible changes that the operation might induce.

While we are not prepared to accept this one experiment as proving that the other organs and tissues are incapable of fixing ouabain, it is difficult to believe that if the vessels in other regions were normally capable of removing the poison from the blood they would have lost that power so nearly completely with the removal of the liver. We believe that this experiment, taken with others, affords corroborative evidence that the liver does play such a preponderant rôle as we have ascribed to it.

The second of these three experiments was designed to show whether excluding the poison from the liver by tying the portal vein exerted any marked influence on its toxicity. Half of the normally fatal dose was injected into the jugular vein of a rat in which the portal vein had been tied. Death resulted within a few minutes. This experiment has many of the objections pertaining to the one previously described.

The third experiment was free from the major objections

urged aganst the other two. It was designed to determine whether a much larger dose of ouabain than the average fatal could be injected into the portal vein without causing death. This animal survived the injection of an amount equal to twice the average fatal dose for a period of nineteen minutes, showing that the poison is less active by the portal, than by the jugular, vein. An amount equal to five times the average fatal dose by the jugular vein was survived for six minutes. This is in striking contrast to the rapidity of the action after the removal of the liver, and also in striking contrast to the amount required to cause death in the previous experiment, in which an even smaller amount would probably have killed the animal. The result of the injection into the portal vein of the rat is also in strong contrast to that seen in the dog, in which there is no perceptible difference in the amounts required to kill whether the injection be made into the femoral, or the portal, vein.

Protocol of experiment 18 (condensed). White rat, weight, 170 grams

10.05 a.m. Liver excised without hemorrhage.

10.25 a.m. 0.5 mgm. ouabain injected into the jugular vein; the respiration ceased in forty-five seconds; heart stopped twenty seconds after the respiration; the animal was exsanguinated.

2 cc. of blood collected from heart and vessels.

1 cc. of blood collected from abdominal region.

The blood was diluted to 50 cc. after defibrination.

Tests

Cat, weight 2.0 kgm.

10.57–11.18 a.m. Injected all of diluted blood intravenously.

11.23–11.57 a.m. Injected 0.025 mgm. ouabain in pure solution; death.

Calculation.

Cat of 2.0 kgm. requires to cause death	0.20 mgm. ouabain
Cat received in pure solution	0.025 mgm. ouabain
Balance in 3 cc. of blood of rat	0.175 mgm. ouabain

Total blood of rat (170 ÷ 16)	10.6 cc.
Loss in liver (not by hem.)	2.6 cc.
	8.0 cc.

If 3 cc. blood contained 0.175 mgm. ouabain, 8 cc. contained 0.47 mgm.

Protocol of experiment (condensed). White rat, weight 166 grams

Portal vein tied during ether anesthesia.

2.47 p.m. 1.0 mgm. ouabain injected into jugular vein (6 mgm. × kgm.). Animal became very sick at once.

2.55 p.m. Heart stopped.

Protocol of experiment (condensed). White rat, weight 162 grams

2.01 p.m. Began the injection of ouabain into portal vein during ether anesthesia.

2.13 p.m. 4 mgm. (24.7 mgm. × kgm.) have been injected; injection interrupted by struggling.

2.16 p.m. Injection resumed.

2.26 p.m. 10 mgm. (61.7 mgm. × kgm.) have been injected.

2.30 p.m. Respiration ceased.

2.32 p.m. Heart stopped.

When the ouabain reaches the duodenum after having passed through the common bile duct its further course is similar to that which arrives at that point after oral administration, but when a small amount, such as may be administered intravenously without causing death, reaches the duodenum a part of it escapes destruction in the large intestine and appears in the feces, although the total amount destroyed in the gastro-intestinal tract after the oral administration of a massive dose may be from ten to nearly one hundred times as great.[8]

In one experiment a little more than 0.5 mgm. of ouabain escaped destruction and was eliminated in the urine and feces after the intravenous injection of a dose of 2 mgm, about 1.5 mgm. having been destroyed, presumably in the large intestine. A

[8] We have called attention (loc. cit.) to a somewhat analogous condition with reference to the destruction of strychnin during putrefaction. Ranke (Arch. f. Anat. u. Phys., lxxix, 1, 1879) administered strychnin to dogs in doses of 100 mgm. and buried their bodies in order to permit of their putrefying during various periods of time. Ranke was unable to detect strychnin by the chemical test after 100 days, but he was able to obtain evidences of its presence by means of the biologic test on frogs even after 330 days. It is difficult to understand why traces of strychnin should resist decomposition for so long a time under conditions which had destroyed vastly larger amounts in less than one-third of the period.

rat received 2.4 mgm. intravenously in another experiment; the feces collected for forty-two hours contained 1.25 mgm. of the poison, or more than half of the total amount injected, which must have passed through the large intestine.

Protocol of experiment (condensed). White rat, weight 210 grams

1.18–1.25 p.m. 2 mgm. ouabain injected into jugular vein; urine collected for twenty hours (first portion); urine collected for next twenty-four hours (second portion); feces collected for forty-four hours.

The urine of the first twenty hours was diluted to 75 cc.

The urine of the next twenty-four hours was diluted to 50 cc.

The feces of forty-four hours extracted to make 50 cc.

Summary of results of tests.

Urine of twenty hours contained	0.173 mgm. ouabain
Urine of next twenty-four hours contained	0.000 mgm. ouabain
Extract of feces contained	0.425 mgm. ouabain
Total amount recovered	0.593 mgm. ouabain

Protocol of experiment (condensed). White rat, weight about 200 grams

3.15–3.25 p.m. 2.4 mgm. ouabain intravenously.

The urine was collected for forty-two hours; diluted to 100 cc.

The feces collected for forty-two hours; extracted to make 200 cc.

Tests

Urine. Cat, weight 1.78 kgm.

10.50–11.25 a.m. Injected all of the diluted rat's urine intravenously.

11.25–11.53 a.m. Injected 0.092 mgm. ouabain × kgm.; death.

Calculation.

Cat weighing 1.78 kgm. fatal dose	0.178 mgm. ouabain
Cat received	0.164 mgm. ouabain in pure solution
Balance due to urine of rat	0.014 mgm. ouabain

(This is obviously within the limits of error).

Extract of feces. Cat, weight, 1.64 kgm.

11.18–12.15 p.m. Injected 15.7 cc. kgm. extract; death.

15.7 cc. contained 0.1 mgm., 200 cc. contained 1.25 mgm.

(A second test was made but discarded owing to error of technic.)

Table 3 shows the doses of ouabain administered; the amounts recovered in the urine and feces (together with the fecal matter in the intestine alone when the period was too short to permit of excretion in the feces); the amounts destroyed, expressed in milligrams, and in percentages of the dose administered; the interval

TABLE 3

Showing elimination of ouabain in urine of rat

WEIGHT	INTERVAL	DOSE	OUABAIN IN URINE	
			Mg.	Per cent of dose
After subcutaneous injection				
grams				
110	24 hours	10.0	1.50	15.0
	24 hours*	10.0	0.16	1.6
297	18 hours	5.0	0.60	12.0
180	18 hours	10.0	1.80	18.0
200	48 hours*	20.0	3.00	15.0
85	24 hours*	8.5	0.55	6.5
210	2 hours	10.0	0.13	1.3
After intravenous injection				
200	10.0 minutes	2.4	0.14	5.8
166	14.0 minutes	2.0	0.14	7.0
202	9.5 minutes	3.0	0.07	2.3
210	20.0 hours	2.0	0.17	8.5
After intravenous injection and tying common bile duct				
210	20.0 hours	2.0†	2.12	106.0
230	20.0 hours	2.0	1.09	55.0
	44.0 hours		1.15	58.0

* These intervals are approximate.

† The bile duct was tied and this dose administered ninety-six hours after a similar dose.

following the administration of the poison during which the urine and feces were collected.

We have found a distinctly smaller percentage of ouabain eliminated by the kidneys after intravenous, than after subcutaneous, injection. We can offer no satisfactory explanation of this,

and it is possible that a larger series of experiments might show less difference than our experiments indicate. The liver is capable of fixing small amounts of the poison more rapidly and energetically than the kidneys, but it is possible that when it has fixed a small amount its capacity for further fixation diminishes until a portion of the poison is eliminated or until it has passed into a later stage of fixation. This would permit of its taking up a larger percentage of an intravenous dose than of the greater amount that can be injected subcutaneously without causing death.

When the ouabain that has been fixed in the liver of the rat, following its intravenous injection, is prevented from passing into the duodenum by tying the common bile duct the poison is redissolved in the blood, carried to the kidneys and eliminated in the urine quantitatively or to a great extent, little being destroyed in the body apparently.

We say that little is destroyed "apparently" because we recognize the fact that it is impossible to determine with certainty by our present method whether very small amounts are destroyed, and it is quite possible that small amounts are destroyed in every case, and much larger amounts in some. It is also possible that small amounts, too small to be detected by us, may be excreted into the intestine in all cases and that under the abnormal conditions of an occluded bile duct this method of elimination may become important, and since destruction in the large intestine may then keep pace with excretion into its lumen we fail to detect it. We believe, however, that we have furnished conclusive evidence that the intestinal epithelium does not excrete notable amounts of the poison under normal conditions.

It is difficult to explain why we have recovered all of an intravenous dose of ouabain from the urine in one case where the bile duct had been tied, and have found only a little more than half of the dose in the urine in another similar experiment except upon some such hypothesis. With less delicate methods than those employed we might well have overlooked the relatively small amounts of ouabain that are normally excreted by the kidneys, just as we now fail to detect any that may be excreted by the

intestinal epithelium in the normal animal. If such small amounts are normally excreted in this way it is probable that with the prolonged sojourn of traces of the poison in the circulation the amounts excreted directly into the large intestine and destroyed there may constitute a large proportion of the total dose.

It may appear like a contradiction of our previous statement to speak of a prolonged sojourn of traces of ouabain in the blood, but it must be borne in mind that we do not claim to be able to detect ouabain in amounts much less than 0.1 mgm., and if as much as 0.001 mgm. is taken up from the liver and eliminated during the period of time required for the blood to make a complete circuit of the intestinal path, the amount eliminated in this way in a few hours is sufficient to account for the difference between the amount administered to the rat and that found in the urine after tying the common bile duct.

The blood is obviously incapable of redissolving more than traces of ouabain, if any, during the time that it is fixed in the cells of the liver and undergoing excretion into the bile ducts, and even when it has passed into the bile ducts the blood takes it up very slowly, so that there are only traces present in the circulation. Since the liver has a much greater capacity than the kidneys for fixing the poison, it follows that the greater part of even these traces must be taken up again by the liver, and only a much smaller part by the kidneys, and possibly by other tissues, and the poison continues to be fixed in the liver again and again in constantly diminishing total amounts until all of it is either excreted or destroyed.

The relative capacities of the liver and kidneys to fix ouabain vary in different individuals, and probably in the same individual at different times, as shown by the varying amounts found in the urine and intestine in different experiments, and consequently the rate of elimination of the poison in the urine after the bile duct has been tied varies widely in different individuals.

This may explain why after tying the common bile duct we found all of the poison in the urine within twenty hours after its injection in one case, and in another such experiment the elimina-

tion in the urine was still in progress after twenty-four hours, in which case the total amount eliminated by the kidneys in forty-four hours was little more than half of that injected intravenously. In this experiment the amount taken up from the liver (including that excreted into the bile ducts) and excreted by the kidneys must have been less than 0.0001 mgm. during the period required for a given red blood corpuscle to make the complete circuit of the liver and kidneys, supposing, of course, that elimination was uniform and continuous. Naturally, this does not refer to the portion of the ouabain fixed in the kidneys immediately after its injection.

Renal activity in the rat depends largely on the amount of food taken when this consists largely of cabbage, and the depression following the operation of tying the bile duct may have influenced the rate of elimination in this time.

It will be observed, however, that in the experiment, the protocol of which is given, the amount of ouabain indicated in the urine was 106 per cent of the total intravenous dose. This animal had received a similar dose ninety-six hours previously, and while it is presumable that all of the first dose had been eliminated, by the liver mainly, it is possible that a small residuum remained in the body. We are inclined to believe, however, that the urine actually contained a little less than the test indicated.

Protocol of experiment. White rat, weight 210 grams

The common bile duct tied and cut during ether anesthesia.

1.03–1.15 p.m. 2 mgm. ouabain injected into the jugular vein.

The urine, 5.2 cc., was collected during a period of twenty-three hours, diluted with normal saline, filtered, and tested on each of two cats in the usual way.

Tests

1. Cat, weight 2.78 kgm.

10.02–10.32 a.m. 3.6 cc. urine (diluted to 75 cc.) intravenously; death.

2. Cat, weight 2.72 kgm.

10.07–10.36 a.m. 3.42 cc. diluted urine as in preceding test; death.

3.51 cc. diluted urine (average) indicate 0.1 mgm. ouabain; 75 cc. contain 2.13 mgm.

Summary

The fatal intravenous dose of ouabain for the rat depends somewhat on the rate of injection, but with the rapid injection of a solution of one part in two hundred parts of normal saline the average is approximately equal to 12 mgm. per kilogram of weight.

Ouabain leaves the blood stream rapidly, and after a few minutes not more than a trace—too small to be detected by our method—is present in the blood of the rat.

Much the larger proportion of an intravenous dose is fixed in the liver at once, or very shortly after the injection, and a smaller part is fixed in the kidney.

It is loosely bound in the vessels of the liver at first (and probably in those of the kidney) and then it may be removed by simple perfusion with normal saline; it appears to pass from the vessels into a more stable combination presumably in the cells of the liver (and in those of the kidney), it passes fairly rapidly from the cells of the liver into the bile ducts and thence into the duodenum; it is extracted easily after it passes into the bile ducts.

After ouabain passes into the duodenum of the rat a variable amount is destroyed in the large intestine, but a part of even so little as may be injected intravenously without causing death, may traverse the intestine and appear in the feces, while equal percentages of many times larger amounts may be destroyed, after the oral administration, in similar periods of time.

When the bile duct is tied, preventing the ouabain from passing from the liver into the duodenum, it is reabsorbed from the liver into the blood, carried to the kidney and excreted quantitatively, or to a very great extent, in the urine.

We have some evidence that a part of the poison injected intravenously is destroyed in the body, probably in the liver.

III. THE ELIMINATION OF AMORPHOUS STROPHANTHIN IN THE RAT

Toxicity of amorphous strophanthin

We planned to conduct a series of experiments with amorphous strophanthin similar to those with ouabain, as we supposed the

toxicity of this substance for the rat to be a little less than that of ouabain by intravenous or subcutaneous injection. This is far from being the case[9] as reference to the tabulated results of our tests on the rat will show.

The average fatal dose of amorphous strophanthin for the rat by subcutaneous injection is about 20 mgm. per kilo, or about a fifth of the amount of ouabain required; the fatal intravenous dose of amorphous strophanthin is approximately 8.5 mgm. per kilo, or about one-third less than that of ouabain.

TABLE 4

Showing the toxicity of amorphous strophanthin for the rat

WEIGHT	DOSE	EFFECT
	Intravenous injection	
grams	*mgm.* × *kgm.*	
52	9.0 (B. & S.)	Death in 7 minutes
44	9.0 (B. & S.)	Death in 7 minutes
52	8.5 (B. & S.)	Recovered
179	8.3 (Merck's)	Death in 8 minutes
92	8.0 (B. & S.)	Recovery after severe depression
52	8.0 (B. & S.)	Recovery after severe depression
	Subcutaneous injection	
150	40.0 (Merck's)	Death in 29 minutes; bile duct tied
130	23.0 (Merck's)	Death within 2 hours
150	20.0 (Merck's)	Recovery, very sick for 7 hours
127	15.6 (Merck's)	Recovery
130	15.4 (Merck's)	Recovery

The average weight of the rats available at the time this investigation was undertaken was about 100 grams, and the amount of strophanthin that can be administered intravenously at once

[9] We used a specimen of Merck's amorphous strophanthin, purchased February 21, 1910, supposed to have been prepared from *S. Kombe*, and a specimen purchased April 21, 1908, labeled "Strophantin chem. pure" which was made by Boehringer and Sons, supposed to be from *S. hispidus*. The biological test 1918 showed that the activity of these specimens had not changed materially, if at all, since their use by Hatcher and Bailey in 1910, the average fatal intravenous dose for cats being 0.187 mgm. per kilo of weight.

to an animal of that size without causing death does not suffice for more than two tests on cats of the average size, weighing 2.5 kgm. It is obvious that we cannot extract small fractions of the intravenous dose of amorphous strophanthin distributed in the organs and tissues of rats of that weight and estimate such fractions by our method, hence we were compelled to modify our plans with reference to the study of its behavior after intravenous injection.

Absorption and destruction of amorphous strophanthin in the alimentary tract of the rat

Amorphous strophanthin, like ouabain, is devoid of toxicity when administered orally to the rat because only a small portion of the oral dose is absorbed, and because excretion keeps pace with absorption, even when delayed peristalsis results in the absorption of unusual amounts.

The behavior of amorphous strophanthin in the gastro-intestinal tract of the rat is closely similar to that of ouabain. Not only does it resist absorption almost completely, despite its solubility, but this lack of absorbability is probably explained in the same way as that of ouabain. At any rate, it is not due to destruction in the stomach and duodenum where absorption would be expected to occur. After a massive oral dose, equal to many times that fatal by intravenous injection, only a small part appears in the feces, in most cases, showing that in the absence of absorption of notable amounts the destruction must take place in the intestinal tract, and we shall see that this destruction actually occurs in the large intestine, as that of ouabain does, and presumably by the same mechanism. A further parallelism with ouabain exists, in that when intestinal peristalsis is interfered with by disturbances of the appetite, and the poison remains in the large intestine for a period longer than that normally required for its passage, the destruction is greater than in those cases where its sojourn in the large intestine is shorter.

We give the protocols of several experiments showing: The absence of destruction of more than negligible amounts of amor-

phous strophanthin in the stomach and duodenum during a period of time more than equal to that normally required for food to pass through this part of the gastro-intestinal tract; the approximate degree to which the poison is absorbed from the alimentary tract under different conditions; and the degree to which it is destroyed after its introduction directly into the duodenum and into the ileum immediately above the ileo-cecal valve.

Protocol of experiment. White rat, weight 115 grams

10.15 a.m. 20 mgm. amorphous strophanthin directly into stomach. No perceptible effect.

2.15 p.m. Animal killed with chloroform.

The stomach and small intestine were extracted in the usual manner, the extract made up to 500 cc. and tested on two cats.

The average of the results of the tests indicated the recovery of 18.7 mgm. of amorphous strophanthin.

The results of this experiment indicate that practically none of the amorphous strophanthin was destroyed during a period of four hours. Had the animal been feeding during this period the poison would have passed beyond the duodenum.

Protocol of experiment. White rat, weight 136 grams

10.20 a.m. 50 mgm. amorphous strophanthin (B. & S.) directly into stomach.

The urine, measuring 30 cc., was collected during a period of seventy-two hours and tested on a cat weighing 1.54 kilograms, which required the injection of all of the urine to cause death. This indicates the presence of 0.29 mgm. of the poison in the urine, or somewhat more than one-half of 1 per cent of the amount administered to the rat.

The feces of the rat were also collected for seventy-two hours, extracted in the usual manner, the extract made up to 100 cc., and tested on each of two cats with closely concordant results, which indicated the presence of 8.24 mgm. of amorphous strophanthin in the fecal extract.

The feces of the rat were then collected for forty-eight hours, extracted, and the extract tested with entirely negative results.

The results of this experiment indicate the destruction of about 85 per cent of the amount administered, and are comparable to those observed in similar experiments with ouabain.

The mere fact that the oral administration of such massive doses (equivalent to about 40 times the fatal vein dose) is not followed by symptoms of any sort indicates that absorption must be very slow or that destruction must take place actively in the organs or tissues of the body, since so little is eliminated in the urine.

Owing to the similarity of behavior of amorphous strophanthin and ouabain in the alimentary tract, thus far observed, we conducted an experiment in which measured amounts were placed in the duodenum of one rat and in the ileum just above the ileocecal valve of another, as in a similar experiment with ouabain. The urine and feces were collected, the feces extracted in the usual manner, and the urine and the fecal extract tested in the usual way.

About 70 per cent of that injected into the duodenum and practically all of that injected into the ileum were destroyed; the total amount excreted in the urine of both rats was about 2.5 per cent of that administered, or relatively five times as much as in the experiment in which the poison was placed in the stomach of a normal animal. The disturbance of appetite from the operation caused delayed intestinal peristalsis, permitting of a longer period of absorption in one of the rats, and a longer period during which it remained in the large intestine of the other, resulting in extensive destruction, as in a similar experiment with ouabain.

It may appear that the difference in the disturbance of appetite and the resulting delay of the poison in the intestine are insufficient to account for the great difference in the degree to which destruction occurred in the two animals, but in the first case the unusual delay occurred while the poison was in the small intestine, and peristalsis was more nearly normal while it was in the large, whereas in the second animal the delay occurred in the large intestine almost entirely.

The similarity of behavior of ouabain and amorphous strophanthin in experiments of this type, and the absence of extensive de-

struction of ouabain in the incubated large intestine to which it had been added, seemed to render such incubation experiments with amorphous strophanthin unnecessary.

Protocol of experiment. White rat, weight 172 grams

1.50 p.m. 15 mgm. amorphous strophanthin (Merck's) into duodenum during ether anesthesia.

4.15 p.m. Animal has eaten little; no feces passed; forty-three hours after operation animal had eaten abundantly and feces were abundant.

Feces of sixty-seven hours collected, extracted, extract made up to 100 cc. Three tests were made on cats, the results indicating the presence in the fecal extract of 4.4 mgm. of the amorphous strophanthin.

White rat, weight 160 grams

2.05 p.m. 15 mgm. amorphous strophanthin (Merck's) injected into ileum immediately above ileo-cecal valve during ether anesthesia. Animal did not eat at all for more than twenty-four hours, no feces passed in that period. Ate abundantly and excreted feces abundantly in forty-six hours; feces collected for sixty-seven hours.

Feces extracted, the extract tested on one cat, weighing 2.5 kgm., which received practically all of the fecal extract, and then required 0.101 mgm. of ouabain per kilogram to cause death, indicating that the extract contained none of the poison.

The mixed urines of these rats were tested on one cat, the results indicating the presence of 0.74 mgm. of amorphous strophanthin.

Elimination of amorphous strophanthin after subcutaneous and intravenous administration

In order to determine approximately the rate at which the rat eliminates amorphous strophanthin from the body we administered toxic, but not fatal, doses which were repeated as often as possible without causing death.

In these experiments we injected amounts equal to 60 per cent of the fatal dose intravenously at intervals of an hour. One animal succumbed to the fourth dose which was administered three hours after beginning the experiment, and one hour after a total

amount equal to 180 per cent of the single fatal vein dose had been given. Since this dose, equal to 60 per cent of the normally fatal, actually caused death, it follows that an amount equal to 40 per cent of the fatal dose remained in effect, hence we must suppose that the animal had disposed of the difference between 40 per cent and 180 per cent, or 140 per cent, in three hours, or the equivalent of one fatal dose in about two hours.

Two other animals survived the administration of four such doses, but they were evidently very near death. We have shown that amounts of ouabain approximately equal to the single fatal vein dose were fixed in the liver of the rat within half an hour after intravenous injection, and this slower elimination of amorphous strophanthin (as indicated by slower recovery) suggests that the poison is eliminated by a different path, or that there is a great difference in the capacity of the liver for fixing the poison and removing it from the blood stream.

Rapid elimination permits of the subcutaneous injection of a much larger dose of ouabain than can be administered intravenously without causing death, but slow elimination results in the accumulation in the blood of a fatal dose after the subcutaneous injection of amounts of amorphous strophanthin more nearly approximating the fatal intravenous dose.

While we have employed these repeated subcutaneous or intravenous injections of a given percentage of the fatal dose as a measure of what we have previously referred to as "essential elimination," the problem is not so simple in every case as it might appear from the frequency with which a given percentage of the fatal single dose may be injected over a short period of time, because the fatal effect of a single dose may be exerted on one structure, and that of repeated doses on a wholly different structure. This shows that the poison exerts a more prolonged action on some structures than on others, and it suggests that the poison is also retained more tenaciously by those organs in which its action persists. Nevertheless, the method does give valuable information regarding the initial rate, as opposed to the completeness, of elimination of many poisons.

The rate of elimination of the greater part of a dose of ouabain

or amorphous strophanthin, is shown in the experiments, but certain structures in the central nervous system of the rat show a persistence of action leading to the accumulation of toxic effects, and we must suppose that these structures retain minute amounts of ouabain and amorphous strophanthin more tenaciously than the heart. The reverse appears to be true of several members of the group with reference to the cat, for the action on the heart of that animal persists much longer than the action on the vomiting center. The center in the rat which shows this persistence of action is concerned with clonic convulsions, which often appear late; the rat usually dies from respiratory paralysis following the intravenous injection of a fatal dose of ouabain or amorphous strophanthin, and this is said to be true of the rabbit also, but we find that cats die from the cardiac effects invariably.

The protocol of an experiment shows that amorphous strophanthin leaves the blood of the rat promptly, not more than traces of the poison being present after the lapse of eight minutes following the intravenous injection of a dose of 1.5 mgm. in a period of twelve minutes. It is quite possible that the blood contained even less than the traces of poison indicated by the test, such amounts being within the limits of error.

Protocol of experiment. White rat, weight 179 grams

12.00–12.12 p.m. 1.5 mgm. amorphous strophanthin (Merck's) into jugular vein.

12.20 p.m. Death.

The rat was exsanguinated by cutting across the heart, after which about 20 cc. of normal saline were injected into the aorta, the escaping fluid was collected and added to the blood, the mixture defibrinated, and tested on a cat weighing 2.4 kilograms.

The cat received all of the defibrinated blood and perfused fluid, and then required 0.089 mgm. of ouabain per kilogram to cause death. All of the blood and perfused fluid, therefore, contained an amount of amorphous strophanthin equivalent in activity to (2.4 × 0.011) 0.0264 mgm. ouabain, or a total of 0.05 mgm. amorphous strophanthin.

We also examined the livers, small intestines, and kidneys with bladders of two small rats following the intravenous injection of

repeated doses of amorphous strophanthin. The protocol of this experiment shows that the small intestines contained a small amount of the poison, the bladders and kidneys half as much as the intestines, and that none could be detected in the livers.

Protocol of experiment. White rat, weight 85 grams

9.37 a.m. 0.425 mgm. amorphous strophanthin (B. & S.) into jugular vein; immediate depression and hyperexcitability.

10.37 a.m. 0.425 mgm. amorphous strophanthin as previously; effects as before.

11.47 a.m. 0.425 mgm. amorphous strophanthin as previously.

12.50 p.m. 0.425 mgm. amorphorous strophanthin as previously.

1.30 p.m. Animal died.

White rat, weight 92 grams

9.51 a.m. 0.74 mgm. amorphous strophanthin (B. & S.) into jugular vein.

10.51 a.m. 0.46 mgm. amorphous strophanthin as previously.

11.52 a.m. 0.46 mgm. amorphous strophanthin as previously.

12.07 p.m. Animal died.

The livers of both rats, weighing together 8.9 grams, were extracted and the extract tested on one cat which received about 70 per cent of the total extract of both livers; the cat then required slightly more than 0.1 mgm. ouabain per kilogram to cause death, indicating that the extract contained no amorphous strophanthin.

The small intestines of both rats, weighing 6.7 grams, were extracted together, the extract made up to 100 cc. and tested on a cat weighing about 3 kilograms; the cat received about half of the total extract of the intestines and then required 0.064 mgm. ouabain per kilogram to cause death; the result indicating the presence of a total of 0.33 mgm. amorphous strophanthin in both rats' intestines.

The kidneys and bladders of both rats were extracted together, the extract made up to 50 cc. and tested on a cat weighing 1 kgm., which received 34 cc. of the extract, and then required 0.042 mgm. ouabain per kilogram to cause death, the test indicating the presence of a total of 0.16 mgm. amorphous strophanthin in the kidneys and bladders of both rats.

The failure to find evidences of the presence of amorphous strophanthin in the livers of these two rats after the intravenous injection of a total of 3.3 mgm. would seem to support the contention of Clark that the liver of the rat does not fix this substance, but its presence in the intestines points to its elimination by the liver. We therefore tied the bile duct of a rat and injected several doses of the poison intravenously at intervals of an hour. The animal died after the lapse of fifteen minutes following the third dose and a total amount equal to about twice the single fatal vein dose.

The protocol of this experiment indicated that the liver contained about one-third of the total amount injected, and while the blood was not examined, the interval elapsing after the injection before death sufficed for the disappearance of practically all of the poison from the circulation, hence there is no question that this poison was actually fixed in the liver and not merely present in the blood in that organ. Furthermore, none of the poison was found in the intestine, a fact that affords support to the view that that found in the intestines in the previous experiment had passed through the liver.

We believe that these experiments also offer an explanation of the greater toxicity of amorphous strophanthin, as compared with that of ouabain, for the rat.

The liver is not only less active in fixing this poison and excreting it, but it probably gives it up to the circulation more abundantly than it does ouabain. Certainly the animal in which the bile duct had been tied succumbed to a smaller total amount relative to its weight than any of the normal animals which received similar repeated doses at similar intervals of time.

Protocol of experiment. White rat, weight, 188 grams

The bile duct was tied during ether anesthesia.

9.45 a.m. 0.9 mgm. amorphous strophanthin into jugular vein extreme depression at once; rapid improvement.

11.00 a.m. 0.9 mgm. amorphous strophanthin as previously; symptoms as before.

12.00 m. 0.9 mgm. amorphous strophanthin as previously.

12.15 p.m. Death; total of 2.7 mgm., or 14.4 mgm. × kgm.

The liver, weighing 9 grams, was extracted, the extract made up to 100 cc. and tested on a cat weighing 2.66 kgm. The cat received 23 cc. of the extract per kilogram, and then required 0.028 mgm. of ouabain per kilogram to cause death. The test indicated the presence of 0.58 mgm. of amorphous strophanthin in the extract of the liver.

The small intestine was extracted and the extract tested on a cat which received more than half of the total amount, after which it required slightly more than the average fatal dose of ouabain to cause death, indicating that the intestinal extract contained no amorphous strophanthin.

We did not observe the influence of tying the bile duct on the toxicity of repeated doses of ouabain, but this operation did not appear to influence the toxicity of single doses, for example, a rat, weighing 123 grams, received an intravenous injection of 2 mgm. of ouabain in a period of nine minutes without causing death during the time which it was designed to have it live (thirty-one minutes); the animal was then killed with chloroform.

The kidneys appear to be capable of eliminating rapidly that part of the ouabain which is taken up by the blood from the liver and which is not fixed in the liver again, as previously mentioned, whereas this reabsorption of strophanthin appears to subject the heart and respiratory center to its continued action to a greater extent than in the case of ouabain.

A rat, weighing 170 grams, was used in such an experiment. The common bile duct was tied during ether anesthesia, after which a dose of 2.6 mgm. amorphous strophanthin was injected subcutaneously; the urine was collected for a period of forty-six hours and tested on two cats, the results indicating the presence of 0.84 mgm. of amorphous strophanthin in the urine. Two rats, weighing 151 grams and 155 grams, respectively, were used as controls of this experiment. The first received a subcutaneous injection of 2.26 mgm., the other of 2.32 mgm., and the urines were collected separately for forty-six hours. The urine of the first rat contained 1.4 mgm., that of the second, 1.5 mgm. of the poison. The dose of amorphous strophanthin used in this experiment corresponds to 15 mgm. per kilogram of weight, and it is

interesting to observe that while the controls recovered promptly, the dose being only about 75 per cent of the average fatal by this mode of administration, the animal in which the bile duct had been tied was very sick throughout the entire period of the experiment, and took no food; another died during the night following a similar dose per kilogram after tying the bile duct. There can be little doubt, therefore, that tying the bile duct does increase the toxic effect of amorphous strophanthin for the rat.

It is to be observed that tying the bile duct does not result in increased elimination of amorphous strophanthin by the kidney as it does with ouabain, in fact, in the experiment just presented only about 33 per cent of the total dose of amorphous strophanthin administered was excreted in the urine by the rat with the bile duct tied, whereas the controls excreted about 65 per cent in that way.

A larger proportion of a subcutaneous dose of amorphous strophanthin than of an intravenous dose is eliminated by the kidneys, as is the case with ouabain. Two rats received a total of 6.8 mgm. of amorphous strophanthin intravenously during a period of three hours; they were chloroformed one hour after the last dose, the small intestines of both animals, examined together, contained 1.4 mgm.; the kidneys and bladders contained 0.32 mgm., or about 5 per cent of the total amount administered. It may be argued that the duration of the experiment was insufficient to permit of the elimination by the kidneys of as much of the poison as in the experiments following subcutaneous injection. It is possible that such an objection may be valid, but the rapidity with which poison leaves the blood following its intravenous injection, together with the rapidity of its passage into the intestine, as previously shown, lead to the belief that very little is fixed in the kidneys after the first few hours. It has been suggested already that absorption from the liver may take place, but the animal disposes of the greater part of a non-fatal dose very rapidly, and it is probable that the amounts which act directly on the heart and respiratory center are far less than those with which we deal in our tests.

In no instance have we been able to recover nearly so large a

proportion of the amorphous strophanthin injected as that of ouabain found shortly after its injection in several experiments. We are unable to explain this satisfactorily. It is possible that amorphous strophanthin is decomposed into the similarly acting, but weaker, strophanthidin, as suggested by Groeber (29) but we have no evidence bearing on this. We do believe, however, that our failure to recover a larger percentage of the amounts injected points to the decomposition of a part of it in the body.

Summary

The behavior of amorphous strophanthin and that of ouabain in the rat present many points of similarity. Both are absorbed very slowly and only to a slight extent from the gastro-intestinal tract, and the portions so absorbed are excreted in the urine in part, and in part by the liver presumably.

They resist the action of the digestive ferments in the stomach and duodenum, but much the larger part of an oral dose is destroyed in the large intestine.

Amorphous strophanthin is much the more toxic by intravenous than by subcutaneous injection in the rat; this difference is apparently due mainly to the slower fixation and excretion by the liver.

A large part of an intravenous dose of amorphous strophanthin is fixed in the liver and excreted into the intestine; the kidneys probably excrete a larger percentage of a subcutaneous, than of an intravenous, dose, and as much as 65 per cent of a subcutaneous dose may appear in the urine. It is possible that a larger number of experiments might show that there is less difference in this respect than we have found.

Tying the common bile duct increases the activity of amorphous strophanthin for the rat, probably because of the reabsorption into the circulation of a part of that which has been fixed in the liver, and which is then prevented from passing into the intestine.

We have been unable to determine with certainty whether any part of an intravenous dose of amorphous strophanthin is destroyed in the liver or other organs of the rat, but such destruction seems probable.

The close similarity in the behavior of ouabain and amorphous strophanthin in the body of the rat renders a more detailed consideration of our experiments with the latter drug unnecessary, because it would be mainly a repetition of what was said concerning the experiments with ouabain.

IV. THE ELIMINATION OF DIGITOXIN IN THE RAT

The recovery and quantitative estimation of digitoxin in extracts of animal tissues and excreta involve far greater difficulties than are involved in similar experiments with ouabain, and we have been unable to follow the course of digitoxin after its administration to rats with the same degree of precision as in the case of ouabain. However, we have investigated its absorption from the alimentary tract; its destruction in the body; its disappearance from the blood; and its excretion, to a limited extent.

The following method of extraction was adopted with some modifications in minor details, dependent upon the nature of the material under investigation and the degree of purification rendered necessary by the presence of fat or other matter in the extract. Blood was defibrinated, diluted with normal saline and used without further treatment; tissues and excreta were ground with sand, treated with dilute alcohol (about 60 per cent) on a water bath, for a few minutes, the extract evaporated on a water bath, the residue taken up in a little dilute alcohol, and this solution used for the biologic test; or the extract was shaken with chloroform, the chloroform distilled, the residue taken up in a little dilute alcohol and the solution tested on cats.[10]

Extracts of the gastro-intestinal tract usually contained a considerable amount of a soap-like substance which we were unable to separate completely from the digitoxin. The extraction of the liver usually presented little difficulty. The amount of 60 per cent alcohol used for dissolving the final residue varied greatly with the amount of material under examination and the anticipated yield of the poison; when it was planned to use only one

[10] When a mixture of three volumes of water and one volume of alcohol is shaken with chloroform, the latter removes nearly all of the alcohol.

TABLE 5

Showing toxicity of digitoxin for the rat

WEIGHT	DOSE	RESULT
		Intravenous
grams	*mgm. per kgm.*	
83	60.0	Fatal at once
197	34.0	Fatal in 1 hour, 41 minutes
190	26.0	Not fatal in 1 hour (chloroformed)
192	26.0	Not fatal in 1 hour (chloroformed)
137	25.0	Fatal in about 3 hours
184	25.0	Fatal at once
165	24.0	Not fatal in 4 hours (chloroformed)
179	20.0	Fatal in about 2 hours, 30 minutes
235	17.0	Fatal in 2 hours, 7 minutes
180	15.0	Fatal in 1 hour, 7 minutes
47	15.0	Fatal in 2 hours, 5 minutes
55	10.0	Fatal in 2 hours, 25 minutes
48	10.0	Fatal in 2 hours, 36 minutes
213	10.0	Fatal in 2 hours, 2 minutes
39	7.5	Normal after 24 hours
55	5.0	Hyperexcitable after 24 hours
		Intramuscular
155	65.0*	Died during night
80	63.0	Fatal in 5 hours, 55 minutes
100	50.0	Fatal after 7 hours, before 22 hours
210	48.0	Survived
229	44.0	Survived, hyperexcitable next day
235	42.0	Survived for 5 hours, 30 minutes
		Subcutaneous
165	120.0†	Fatal after 6 hours, 30 minutes (during night)
87	115.0‡	Survived for 24 hours (chloroformed)
56	89.0	Fatal after 26 hours, before 40 hours
236	42.0	Apparent recovery, death after 7 days
125	40.0	Survived

* Two doses of 32.5 mgm. × kgm. each, with interval of 1 hour.

† Fatal after more than 6 hours, 30 minutes following a second dose of 60 mgm. × kgm. with an interval of 2 days.

‡ Two equal doses with interval of 2 hours, 13 minutes.

TABLE 5—*Continued*

WEIGHT	DOSE	RESULT
		Oral
grams	*mgm. per kgm.*	
75	133.0	Died during night
39	125.0	Died during night
41	100.0	Died during night
55	75.0	Fatal after 30 hours, before 47 hours
103	49.0	Fatal after 48 hours
106	47.0	Fatal on fifth day
127	40.0	Fatal after 4, before 6 days
143	35.0	Survived
145	34.5	Survived
145	34.5	Survived
150	33.3	Survived

cat as the test animal not more than 5 cc. of dilute alcohol was used. Our object was to avoid purification as far as possible, since the necessary manipulation nearly always involves loss of active principle, and, as previously stated, extracts of rat's tissue and excreta can be injected intravenously into cats without interfering with the quantitative estimation of the digitalis bodies held in solution.

The average fatal intravenous dose of digitoxin for the cat is 0.35 mgm. per kilogram of weight, but it shows greater variation than ouabain for different individuals. The action of the minimal lethal dose is elicited more slowly than is that of ouabain, consequently with continuous administration there is a longer interval after the fatal dose has been injected before the typical symptoms of a fatal effect are induced, and the excess over the amount actually required is greater than with the more rapidly acting ouabain, involving a correspondingly greater error in the estimation of the amounts present in the fluid to be tested. This error in the estimation of digitoxin can be lessened in some cases by employing the "combined ouabain" method previously mentioned.

With the slow and continuous injection of a suspension of digitoxin in normal saline the fatal dose for the cat is about 0.5 mgm.

per kilogram, when the injection is made at such a rate that about an hour and a half are required for the administration of that amount of the poison, but the fatal dose by the "combined ouabain" method is about 0.35 mgm. per kilogram of weight.

While we give the results in figures that suggest extraordinary precision, the error involved in this series of experiments is almost certainly greater than that in the corresponding experiments with ouabain, nevertheless, it is not of sufficient degree to vitiate the conclusions drawn.

The average fatal intravenous dose of digitoxin for the rat is approximately 8.5 mgm. per kilogram of weight, but some animals survived for several hours the intravenous administration of amounts equal to two or three times the average minimal fatal vein dose. The minimal fatal dose by the vein is about equal to that of amorphous strophanthin and is materially less than that of ouabain. The fatal oral dose of digitoxin for the rat is about equal to that by subcutaneous injection, being approximately equal to 45 mgm. per kilogram. We have been unable to determine the exact average fatal dose of this poison for the rat by the several modes of administration without employing a larger number of animals than we felt justified in using. The figures given in the table showing toxicity are taken from various experiments in some of which the animals were killed after an interval of time previously selected, and which serve at least to show that the doses used were not more rapidly fatal.

Absorption and destruction of digitoxin after oral administration

A comparison of the effects seen after oral administration with those following intravenous injection affords a rough measure of the absorption which occurs within a period of a few hours, the action of digitoxin being so persistent that it is relatively unimportant in this respect whether a given amount is absorbed in two hours or in twice that period. A much larger total amount, however, may be absorbed within a day, during which time elimination almost keeps pace with absorption, for the table shows that amounts equal to two or three times the fatal intravenous

dose may be injected subcutaneously without causing death, and we must suppose that practically all of a subcutaneous dose eventually passes into the circulation.

The figures given in the table indicate the extreme variability in the absorption of digitoxin from the gastro-intestinal tract of the rat. The absorption is almost certainly influenced by the rate at which the poison traverses the intestine, and when that portion which has been absorbed gives rise to nausea and loss of appetite, with resulting diminished peristalsis, the poison takes longer to pass the small intestine, permitting of increased absorption. This probably results in the establishment of a vicious circle, and it may explain the fact that some of our rats were more or less continuously sick for periods up to five days following the oral or subcutaneous injection of a single dose of digitoxin, in which case the poison was probably slowly absorbed and excreted to a large extent.

We have attempted to determine approximately the percentage of the oral dose which escapes destruction and appears in the feces. In one experiment of this series practically all of the digitoxin administered was recovered in the feces; in another experiment the amount of the poison recovered was roughly one half of that administered; in two others we were able to recover only an insignificant fraction of the oral dose.

There is no reason to doubt that in some experiments very little of the poison was destroyed and that in others very little escaped destruction, but the results of these experiments give no clear indication concerning the seat of destruction. It is possible that a part of the absorbed poison is destroyed in the liver.

Elimination of digitoxin after intravenous and subcutaneous injection

Lhoták reported, as previously stated, that digitoxin leaves the blood of the rabbit almost immediately, but we found evidence of its presence in the blood of two rabbits one of which lived forty-nine minutes after an intravenous injection, the other having been killed by a medulla stroke ten minutes after the intra-

venous injection of an amount that was not much more than the average minimal fatal dose.

We have also examined the blood of rats after various intervals of time following the intravenous injection of digitoxin. None of the poison could be detected in the blood of two rats which were killed one hour after the intravenous injection of 5 mgm. into each. Not a trace of digitoxin could be detected in the blood of a rat which was exsanguinated five minutes after the intravenous injection of 4.15 mgm. The protocol of another experiment shows that nearly all of an intravenous dose of digitoxin had left the blood stream in five minutes, at which time the liver had certainly fixed a small amount.

Protocol of experiment. White rat, weight, 228 grams

12.07–12.08 p.m. 5 mgm. digitoxin (Merck's) 1–200 dilute alcohol and N.S. into jugular vein.

12.13 p.m. Exsanguinated, 10 cc. blood obtained, a little normal saline used to wash vessels.

White rat, weight 189 grams

12.30–12.31 p.m. 5 mgm. digitoxin injected as in preceding.

12.36 p.m. Exsanguinated as in preceding, 5 cc. blood obtained.

The mixed blood of both rats was defibrinated and diluted with enough normal saline to make 50 cc.

The livers of both rats were extracted, and the extract made up to 5 cc. with dilute alcohol.

Tests

Diluted blood.

Male white cat, weight 2.48 kgm.

2.10–3.35 p.m. Injected all of the diluted blood; began injection of ouabain 1–100,000.

4.12 p.m. Convulsions; total of 0.066 mgm. ouabain injected.

Since 0.066 mgm. ouabain is fatal to 0.66 kgm. of cat, the digitoxin in the diluted blood corresponds to the fatal dose for (2.48 − 0.66 =) 1.82 kgm., or (1.82 × 0.35) 0.63 mgm. in 15 cc. of blood. According to the tables previously cited, the total amount of blood in these two rats would correspond to (228 + 189 = 417. 417 ÷ 16 =) 26 cc., which would contain 1 mgm. of digitoxin.

Extract of liver.

Male cat, weight 2.86 kgm.

1 cc. of the extract was injected intravenously every twelve minutes until 4 cc. had been injected, and twelve minutes later the remainder of the available extract—0.7 cc.—was injected. Five minutes later the injection of ouabain 1–100,000 was begun and after twenty-nine minutes convulsions and death resulted, when a total of 0.1 mgm. had been injected. Since this amount of ouabain is fatal to 1 kgm. of cat, the extract of the livers must have contained enough digitoxin to kill 1.86 kilograms, indicating the presence of 0.65 mgm. in 5 cc. of the extract.

The urines of rats were examined after various intervals following the administration of digitoxin by the mouth, subcutaneously, and intraperitoneally, but no evidence of its excretion by the kidney could be obtained in the normal animal. Three rats received a total of 20 mgm. of digitoxin (15 mgm. to two intramuscularly, 5 mgm. to one intraperitoneally) the urines were collected for periods varying from seven to twenty-four hours, evaporated, the residue extracted with dilute alcohol and the extract tested on frogs with entirely negative results.[11]

Traces of digitoxin were indicated by the test of the urine of a rat collected for four days following the subcutaneous injection of a total of 10 mgm. after the bile duct had been tied. The amount indicated—about 0.25 mgm.—lay within the limits of error, and it is probable that not even a trace of digitoxin was present in the urine.

The feces of this animal were collected during a period of six days, extracted, and the extract tested in the usual manner, though it seemed improbable that the poison could be excreted in the feces after the bile duct had been tied. The results of this experiment, the protocol of which is given, leave little doubt that nearly all of the digitoxin was destroyed in the body of the rat, and the fact that the animal recovered almost as soon as the normal animal following similar doses, despite the fact that its bile duct was tied, points to the liver as the probable seat of destruction.

[11] The urine of one rat, which received a total of 10 mgm., was collected for twenty-four hours, that of one, which received 5 mgm. intramuscularly, for about seven hours, the data concerning the collection of urine from the one that received 5 mgm. intraperitoneally are lacking.

Protocol of experiment. White rat, weight 236 grams

The bile duct was tied during ether anesthesia.

2.00 p.m. 5 mgm. digitoxin by vein, in 1–200 alcohol and normal saline.

2.15 p.m. 5 mgm. digitoxin by vein as previously.

The animal ate little during the next three days; on the fourth day after injection it had eaten fairly well.

Death occurred after seven, and before the end of nine days, the animal having eaten well and passed abundant feces before death.

The urine was collected for ninety-six hours, diluted to 25 cc. and tested in the usual manner; the feces were collected up to the time of death, extracted as usual, the extract made up to 15 cc. and tested.

Tests

Urine.

Female cat, weight 2.26 kgm.

2.52–3.00 p.m. Injected all of urine intravenously.

3.10 p.m. Began injection of ouabain, 1–100,000.

3.47 p.m. Convulsions, death; total of 0.151 mgm. ouabain.

Calculation.

Cat required equivalent of	0.226 mgm. ouabain to kill
Cat received	0.151 mgm. ouabain
Difference	0.075 mgm. ouabain

0.075 mgm. ouabain, equivalent of (0.075 × 3.5) 0.26 mgm. digitoxin.

Fecal extract.

Female cat, weight 2.2 kgm.

1.53–2.38 p.m. Injected total of 8.8 cc. extract.

2.56 p.m. Began injection of ouabain, 1–100,000.

3.23 p.m. Convulsions, death; total of 0.114 mgm. ouabain.

The calculated digitoxin content of the total fecal extract was 0.63 mgm.

Numerous efforts were made to determine whether digitoxin is fixed in the liver and excreted in the bile after the intravenous or subcutaneous injection of the poison, but we have been unable to recover more than a small portion of the amount injected in any case. Absorption from the subcutaneous tissue takes place slowly and elimination almost keeps pace with absorption unless a mas-

TABLE 6

Showing recovery of digitoxin from body or feces

WEIGHT	DOSE	INTERVAL	RECOVERED	WHERE FOUND
		After intravenous administration		
grams	*mgm.*		*mgm.*	
165	4.0	4 hours, 5 minutes	1.5(?)	Intestine
197	6.7	1 hour, 40 minutes	1.4	Intestine
192	5.0	1 hour	0.0	Liver, small intestine
190	5.0	1 hour		
137	3.4	* 3 hours	1.0	Liver, small intestine
179	3.6	* 2 hours, 30 minutes		
189	2.7	* 2 hours		
		After intramuscular or subcutaneous injection		
87	10.0†	24 hours	0.6	Intestine and feces
75	10.0‡	20 hours, 30 minutes	Traces	Intestine and feces
200	10.0§	2 days	Traces	Intestine and feces
56	5.0	26–40 hours	0.0	Intestine and feces
125	5.0	5 days	0.8(?)	Feces
80	5.0	6 hours	0.0	Intestine
210	10.0	23 hours	1.6	Intestine and feces
235	10.0	5 hours, 30 minutes	0.8	Intestine
100	5.0	7 hours, 22 minutes	Traces	Intestine
235	4.0	2 hours	0.6	Intestine
		After oral administration		
143	5.0	6 days	5.0	Feces
145	5.0	4 days	2.5	Feces
145	5.0	5 days	0.5	Feces
150	2.0	2 days	0.0	Feces
150	3.0	2 days		

* Intervals are approximate.

† Two doses of 5 mgm. each with an interval of 2 hours, 13 minutes

‡ Two doses of 5 mgm. each with an interval of 1 hour, 13 minutes.

§ Two doses of 5 mgm. each with an interval of 25 hours.

sive dose has been given, and this may account for our failure to find it in the liver after the subcutaneous injection of large doses. In one experiment three rats received a total of 9.7 mgm. of digitoxin by intravenous injection; two died in about two hours and one in about three hours; the biological test of an extract of the livers and small intestines of all three indicated the presence of about 1 mgm. of the poison. If the liver excretes digitoxin into the small intestine the excretion must take place slowly, and this may account for our failure to find even traces of the poison in the intestine in some experiments in which fairly large amounts had been given, whereas in other experiments we obtained unmistakable evidence of its presence in the intestine following its subcutaneous injection.

Table 6 shows the amounts of digitoxin administered to rats; the several modes of administration; the intervals before death or during which the urine or feces were collected; and the amounts of digitoxin recovered. A question mark is placed after two of these amounts to indicate that the tests were not wholly satisfactory. In one of these the injection of all of the available extract failed to cause perceptible effects after which the animal required an amount of ouabain nearly equal to the average fatal dose before death resulted. It is obviously impossible in such a case to determine whether the extracts contained traces of digitoxin.

Summary

The following refers only to the behavior of digitoxin in the rat.

Digitoxin is absorbed from the gastro-intestinal tract far more readily than ouabain or amorphous strophanthin, though it is practically insoluble in water.

The proportion of an oral dose of digitoxin that escapes destruction and appears in the feces varies widely in different experiments. Nearly all is destroyed in some, very little in others. This destruction is probably greater when loss of appetite prevents active intestinal peristalsis permitting a longer sojourn of the poison in the intestine.

Much the larger part of an intravenous dose of digitoxin—90 per cent or more—leaves the blood within a period of five minutes.

Part of the digitoxin injected intravenously is fixed in the liver, at least a part reaches the intestine, and a part may even appear in the feces. Much the larger part of a severely toxic intravenous dose is destroyed in the body.

Digitoxin is not excreted by the kidneys of the normal rat except possibly in traces, and it is possible that somewhat larger amounts may appear in the urine after the bile duct is tied, but this remains to be determined.

The action of a single toxic oral, subcutaneous, or intravenous dose of digitoxin is far more lasting than that of ouabain, but a very large amount of digitoxin may be survived following its subcutaneous injection in two doses administered within a short time.

The toxicity of digitoxin for the rat is about the same as that of amorphous strophanthin by intravenous injection, it is far the more toxic with oral administration; far less with subcutaneous injection.

These differences in the toxicity of the two poisons by different modes of administration depend on differences in their rates of absorption (except after intravenous injection) and elimination.

V. THE ELIMINATION OF DIGITALEIN IN THE RAT

Pure digitalein is not readily available and the commercial (crude) product is said to consist of a mixture of principles, including digitoxin, digitalin, anddigitalein.[12] It is generally stated that digitoxin is rendered soluble in water by the presence of digitonin, and this commercial digitalein is readily soluble in water, affording a solution which froths on shaking.[13]

It seemed hardly worth while to conduct an extensive investigation with an impure product, such as this, but we have per-

[12] New and Nonofficial Remedies, 1918, p. 100.

[13] Five milligrams each of digitalein and digitoxin were triturated with 25 cc. of normal saline; the resulting milky looking mixture was filtered clear and the filtrate tested on two cats. The results indicated that about 40 per cent of the digitoxin had passed into solution.

formed a few experiments and while the results of these are not especially promising they have a certain value.

Some preliminary experiments with digitalein on the rat were made by Mr. (now Professor) M. I. Smith, at that time a student in this laboratory. These experiments were conducted under the supervision of one of us (Hatcher) who assumes responsibility for the results obtained. Doses of 50 or 100 mgm. (total) were injected intramuscularly, and after periods varying from thirty to seventy-five minutes the tissues, exclusive of skin and intestine, were extracted with normal saline in the manner described for the extraction of ouabain; the extracts were tested on cats in the usual way; the results indicated the recovery of 40, 45, and 51 per cent respectively, of the amounts injected.

The urine was collected for periods of 16.5 and 20.5 hours, respectively, after the intramuscular injection of doses of 100 mgm. (total) in each of two experiments, and the urine and feces were collected and tested daily for three days after the injection of a dose of 50 mgm. In no case was the poison detected in the urine or the feces.

While digitalein is soluble in water, one should expect its separate constituents to behave independently after they have entered the blood and therefore in some experiments, made subsequent to those just mentioned, we have extracted the tissues and excreta with normal saline for the recovery of the water-soluble fraction and have then extracted the residue in the manner described for the extraction of digitoxin. Blood has been tested directly after simple dilution with normal saline. The protocol of a control experiment follows:

Ten to 20 cc. of blood were drawn from a cat into a dish containing 100 mgm. of digitalein dissolved in a few drops of normal saline, the mixture defibrinated, diluted with normal saline to make 300 cc., the proteins precipitated by heating on a water bath, the mixture filtered, and the resulting opalescent filtrate tested on cats.

The results indicated the recovery of practically all of the digitalein.

The residue left on the filter was expressed, extracted with 100 cc. of 60 per cent alcohol, the alcohol evaporated, and the residue extracted with chloroform, the liquids being allowed to stand over night. The

chloroform was evaporated and the residue dissolved in 5 cc. of 60 per cent alcohol. A test of this on a cat showed the recovery of an amount of digitalein corresponding to that contained in the fluid remaining in the residue after expression.

Two hundred cc. of the original extract (remaining after the tests) were shaken with chloroform and allowed to stand over night. Fresh chloroform was added to the aqueous layer and allowed to stand for two days; the mixed chloroformic extracts were evaporated and the residue dissolved in 25 cc. of a mixture of normal saline and alcohol, which was tested in the usual manner.

The test indicated that the chloroformic extract contained about 8 mgm. of digitalein, or since digitalein is insoluble in chloroform, we should say that it contained the equivalent in toxicity of that amount of digitalein, in the form of a chloroform-soluble substance—probably digitoxin.

The fatal intravenous dose of digitalein for the cat is about 1.5 mgm. per kilogram, that for the rat is approximately 75 mgm. per kilogram, but individual rats show rather wide differences in tolerance; the intramuscular injection of amounts corresponding to about 600 mgm. per kilogram was survived until the animals were chloroformed after intervals of thirty minutes, one hour, and sixteen and a half hours, respectively, while one such dose was not fatal during the period of observation. The oral administration of slightly larger doses proved fatal to each of two animals after periods of about thirty hours, but one animal survived an oral dose of 725 mgm. per kilogram.[14]

[14] The digitalein used by us was obtained in bottles, each containing a gramme or less, and all bearing the label of Merck. It was not feasible to determine the activity of each specimen, and we did not take the precaution of recording the particular bottle from which the digitalein was taken for each experiment.

When the investigation was nearly completed we found a specimen that was about twice as active as we supposed it to be. This specimen shows a fair degree of uniformity of activity when tested on the cat, except that two cats used in a series of six, died with such small doses that we must attribute their deaths to that part of the digitalein which is not a true digitalis principle. In nearly all of the experiments in which its synergism with ouabain was tested it behaved like other digitalis bodies.

A comparison of the results following its intravenous injection into rats with those observed when other specimens were used, leads us to believe that all of the digitalein used, except that employed in the earlier experiments, was of approximately the same degree of activity.

Destruction of digitalein after oral administration

Three rats, weighing 105, 131, and 137 grams, respectively received doses of 100 mgm. (total) of digitalein each, directly into the stomach. All were sick on the following day, the symptoms resembling those seen after digitoxin. The two smaller, rats died after an interval exceeding twenty-seven hours, the other one recovered.

The urine and feces of the surviving rat were collected during a period of four days, extracted together, and the extract tested on a cat by the "combined" method. The test indicated the presence of about 2 mgm. of digitalein in the total extract, but it is possible that none was present, as only about one-third of this extract was used in the test, after which the animal required 71 per cent of the average fatal dose of ouabain to cause death. A second test was not made as it was deemed of little importance whether a milligram more or less escaped destruction after the oral administration of such a large dose.

This result agrees fairly closely with those observed by Mr. Smith in the earlier experiments in which the digitalein was injected intramuscularly.

Elimination of digitalein after intravenous injection

The results of the following experiment show that digitalein, or those constituents which have a true digitalis action, leave the blood of the rat fairly promptly after intravenous injection. Two rats received intravenous doses of 8.2 mgm., and 9.4 mgm., of digitalein, respectively (corresponding to doses of 85 and 100 mgm. per kilogram). The first was exsanguinated after fifteeen minutes, the second after ten minutes, the blood of each was defibrinated, diluted with normal saline, and tested on cats. All of the available blood in each case was injected into a cat, after which it required the full average fatal dose of ouabain to cause death, showing that no true digitalis principle was present in the defibrinated blood of the rats. Both of the cats used in these tests voided bloody urine just before death, this being almost certainly the result of the laking action of the digitonin

present in the digitalein. A small amount of blood was removed from the heart of one of these cats, diluted with normal saline, and allowed to stand until the corpuscles had fallen to the bottom of the vessel. The supernatant fluid was of a faint red color, showing that a slight degree of hemolysis had occurred.

The livers of these two rats were extracted, first for water-soluble principles, then for digitoxin, and the extracts tested on cats, but no evidence of the presence of any substance with a digitalis action could be obtained, the two cats took all of the extracts, and then each required the full average fatal dose of ouabain to cause death.

In another experiment a rat, weighing 174 grams, received an intravenous injection of 17.4 mgm. (total) of digitalein. Death resulted in three minutes; the animal was exsanguinated with the injection of a little normal saline, the blood was defibrinated, diluted with normal saline, and tested on two cats with fairly concordant results which indicated the presence of 8 mgm. of digitalein in the blood of the rat, or about half of the amount injected.

In a third experiment a rat weighing 98 grams received a total of 7.5 mgm. of digitalein. Death resulted in six minutes; the diluted, defibrinated blood was tested on two cats; one of these died by respiratory paralysis, which is never the cause of death when true digitalis principles are used in this way. This test, if accepted, would indicate the presence in the blood of about as much digitalein as we injected. Death was, however, probably due to the digitonin constituent. The other test animal received all of the remainder of the rat's blood—about twice as much as the first—after which it required somewhat more than the average fatal dose of ouabain to cause death, which occurred typically. We consider that the results of these tests afford conclusive evidence that any true digitalis principle (not including digitonin) present in digitalein leaves the blood of the rat fairly rapidly after its intravenous injection, but they afford no data concerning the length of time required for the digitonin constituent to leave the blood.

While we had no evidence that the liver plays any rôle in the

elimination of digitalein, we thought that we might obtain indirect evidence of its capacity for fixing and excreting the poison by tying the bile duct and examining the urine after injecting digitalein. The bile duct of each of two rats was tied during ether anesthesia, after which a total of 15.5 mgm. of digitalein was injected intravenously. The urine and feces were collected for a period of four days, extracted, and the extract tested on a cat, which received about half of the total extract, and then required the average fatal dose of ouabain to cause death, indicating that neither the urine nor the feces contained any true digitalis principles. We must suppose, therefore, that these principles are almost completely destroyed in the body after the oral, intravenous, or intramuscular administration of digitalein.

Summary

The fatal intravenous dose of the most active available commercial digitalein for the rat is roughly 75 mgm. per kilogram; that by oral or subcutaneous injection is nearly ten times as much.

Those constituents of digitalein that exert a true digitalis action leave the blood of the rat promptly, the digitonin constituent probably disappears much more slowly.

We have no evidence that the constituents of digitalein having a true digitalis action are fixed in the liver of the rat, but the question remains open.

Not more than traces of the true digitalis bodies of digitalein appear in the urine or feces after oral, intravenous, or intramuscular administration, hence they must be destroyed in the body.

We have not determined the seat of this destruction in the normal animal, but since it occurs after the bile duct has been tied, it seems probable that the liver is the chief organ concerned in the destruction of that portion which enters the circulation; the large intestine is probably concerned with the destruction of the greater part of an oral dose.

VI. THE ELIMINATION OF OUABAIN IN THE CAT AND DOG

The behavior of ouabain in the cat is so nearly like that in the dog that experiments on these two species will be considered to-

gether. The similarity of the behavior of several of the digitalis principles in the cat in man, especially with reference to their absorption from the gastro-intestinal tract, their relative toxicity after different modes of administration, the seat of their emetic action, and their persistence of action on the heart, suggests that man eliminates these principles in much the same way as the cat, hence we have made many efforts to secure convincing evidence of the behavior of the more important members of the group in the cat, but we shall here limit our discussion to the behavior of ouabain because we have been unable to devise any satisfactory method of investigating the behavior of digitoxin or other members of the group.

The want of a test animal that stands in the same relation to the cat or dog that the cat does to the rat has prevented the use of methods similar to those employed in the experiments on that animal without extensive modification, we have, therefore, resorted to the use of massive doses in many of the experiments. This affords an abundance of material for extraction, but the animals die so promptly that we are unable to determine the ultimate fate of the poison. If, on the other hand, we use small doses it is impossible to recover enough of the poison in the extracts of excreta in a form suitable for testing.

Several phases of the problem have been attacked in different ways with some overlapping in the experiments and this prevents an entirely systematic presentation of this part of the work, as that would entail duplication.

The average fatal dose of ouabain for the dog is slightly larger than that for the cat in proportion to the weight, whether the injection be made subcutaneously or intravenously, the amount required for either animal by subcutaneous injection being about one-fourth larger than that required by vein, even though the intravenous injection be extended over a period of several hours.

Since the greater part of that injected subcutaneously is absorbed within an hour or less, it is difficult to understand why so much more is required by this method of administration. One can hardly suppose that the small amount of subcutaneous tissue with which it comes in contact is capable of destroying enough of

the poison to account for this difference, and, on the other hand, it is difficult to understand how one-fifth of a subcutaneous dose can resist absorption after four-fifths have been absorbed promptly.

Absorption from the gastro-intestinal tract is so variable in both species that one can say little more about it than that the oral administration of aqueous solutions of amounts equal to five to ten times the fatal intravenous dose is usually fatal. There can be no doubt that the lower toxicity of ouabain by oral administration is due mainly to the characteristic resistance to absorption in the stomach and small intestine, seen in all species of animals (including man) in which we have studied this problem, but we have no data concerning its destruction in the large intestine of any animal except the rat.

The elimination of ouabain from the blood of the cat and dog[15]

We have extended the observation of Hatcher (27) on the rate of disappearance of ouabain from the blood of the cat and have confirmed his statement that a large part of an intravenous dose leaves the blood within a few minutes.

We have injected ouabain intravenously in the cat and dog in amounts varying from 5 to 140 times the fatal vein dose and have determined the amounts remaining in the blood at death. Death results usually in about three minutes when several times the minimal fatal vein dose is injected, and the interval before death is not materially less when an amount equal to many times the minimal fatal dose is administered.

After the injection of amounts equal to about 100 times the minimal fatal vein dose the blood, at death, usually contains about one-half to two-thirds of that injected; a somewhat smaller proportion of a still more massive dose was found in the blood in one experiment, in which the heart stopped in two minutes and twenty-three seconds; the amount found in the blood after in-

[15] The cat has been used in much the larger number of our experiments of this series but we believe that the similarity of their behavior toward this poison in parallel experiments justifies our including the dog with reference to the conclusions drawn.

jections of approximately ten times the fatal vein dose averaged about one-third of that injected.

Table 7 shows the amounts of ouabain injected; the amounts found in the blood; the interval following injection before death; and the percentage of that injected which had left the blood.[16]

TABLE 7

Showing the rate of elimination of ouabain from the blood of the cat

AMOUNT		INTERVAL		ELIMINATED
Injected	Found in blood	Minutes	Seconds	
mgm.	*mgm.*			*per cent*
1.35	0.61	2	0	55
3.10	0.73	2 (about)		77
3.26	0.63	2 (about)		81
5.52	1.90	2	10	65*
7.50	1.57	2	43	79
25.00	8.10	2	40	68
25.00	9.50	1 (about)		62
25.00	7.70	2	30	69
25.00	8.90	2	23	64
25.00	9.40	2	0	62
25.00	7.70	2	0	69
37.50	15.20	2	30	60
50.00	27.40	2	23	45

* Practically all of the blood of this animal was obtained by exsanguination and subsequent perfusion.

The fixation of ouabain by the liver of the cat and dog

The rapidity with which ouabain leaves the blood indicates that it is fixed by some tissue or tissues with which the greater part of the blood comes in contact during a period of less than two minutes. The several tissues which individually meet this requirement are those of the liver, heart, lungs, brain, volun-

[16] We have based our calculations on the estimate, now generally accepted by physiologists, that the blood of the cat is equal to 5 per cent of the body weight. It was formerly stated that the blood represented 7 per cent of the weight. The acceptance of this figure would indicate the recovery of a larger amount of ouabain in the blood than is shown in the table, but it is of little practical importance whether the amount of ouabain remaining in the blood after five minutes is 30 per cent or 40 per cent of that injected.

tary musculature, gastro-intestinal tract, and perhaps the kidneys. We have investigated these successively in the effort to determine the part which each plays in removing the poison from the blood. For convenience the blood vessels are considered as a part of the tissues which they supply and from which they cannot be separated, but we have endeavored to learn whether they fixed ouabain during perfusion with it.

The observation that the liver of the rat plays such an important rôle in the elimination of ouabain inclined us to the view that this organ is also concerned in its elimination in other species of animals, but, on the other hand, it is plain that the liver of the cat or dog is incapable of protecting it against such large doses as the rat withstands, hence we did not expect to find the poison fixed in the liver in large amounts.

Massive doses of ouabain were injected intravenously into cats, the animals were exsanguinated at death, or within a few minutes thereafter, the liver, previously rendered nearly bloodless in some cases, was extracted and the extract tested on cats in the usual manner.

None of the poison was recovered from the liver in some experiments; in others small amounts were recovered, but in no instance were these sufficient to account for more than a fraction of that which had left the blood.

The protocol in brief of one of these experiments follows.

A cat, weighing 3.94 kgm, received 25 mgm. ouabain into the femoral vein; the heart stopped after two minutes; the portal vein, and the vena cava above the liver, were tied; the liver was removed and drained of blood; this, and that drained from the remainder of the body, were defibrinated separately, each was diluted with normal saline and tested in the usual manner.

After the blood had been drained from the liver it was perfused with enough 2 per cent solution of sodium chlorid to furnish 200 cc. of perfused fluid; it was then perfused with normal saline until 280 cc. of the second perfused fluid were obtained. This second perfused fluid was almost colorless.

The liver, weighing 75 grams, was extracted in the usual manner with 300 cc. of normal saline, and the several fluids were separately tested.

The results of the tests indicated the presence of the following amounts of ouabain:

Total blood of the body	8.40 mgm.
Blood drained from liver, 7.5 cc.	0.35 mgm.
First perfused fluid from liver, 200 cc.	0.92 mgm.
Second perfused fluid from liver, 280 cc.	0.30 mgm.
Extract of liver, 375 cc. (including liver tissue)	1.25 mgm.

It is sometimes stated that the liver contains about one-fourth of the total blood of the animal, however, we were able to obtain only 7.5 cc. by simple drainage in this experiment, but practically all of the remainder was removed by perfusion with the first portion of 200 cc. of fluid, which yielded approximately 1 mgm. of ouabain; the second perfused fluid contained much less than one-third as much blood as the first, since it was nearly colorless, but it contained about as much ouabain as the 7.5 cc. of pure blood, and we must suppose that a portion of this amount of the poison was removed from the liver cells or from the blood vessels of that organ. The liver tissue was then practically bloodless, but the extract contained 1.25 mgm. of ouabain.

It may be seen from the summary of results of this experiment that the total amount of ouabain recovered from the liver, including that from its blood, the first and second perfusions, and the extract, was 2.82 mgm., an amount almost precisely equal to one-third of that indicated in the total blood of the animal. This would seem to confirm the view that the liver did contain one-fourth of the total blood and that nearly all of the ouabain recovered from that organ was present in the contained blood, but the fact that the second perfusion fluid, though almost entirely free of blood, contained about as much of the poison as the 7.5 cc. of pure blood drained from the liver, and the further fact that the practically blood-free liver contained as much of the poison as this pure blood and the first perfused fluid together disprove any such contention. A liver weighing 75 grams could not retain half of its blood after having been drained as far as possible and then perfused until the fluid was nearly colorless, nor could it yield a nearly colorless extract with so much blood present.

We found the concentration of the poison to be the same in the blood of the liver and that of the remainder of the body in two of the experiments in which comparative tests were made. It might be argued from this that the liver does not fix the poison. However, the greater part of the blood of the cat passes through the liver several times within a period of two minutes and we have no means of detecting minute differences in concentration of the poison, such as must exist in the blood of different regions; this similarity in concentration of the poison in the blood of different parts of the body suggests its fixation by more than one organ or tissue (cf. results of experiments on rats). In one experiment the concentration in the blood of the liver was much less than that in the remaining blood.

We thought at times that we failed to recover from the liver all of the ouabain present; we therefore resorted to feeding experiments in the belief that any stable combination of the ouabain with the liver cells would be broken down during digestion and the ouabain then absorbed. We did not expect to be able to estimate accurately the amount of ouabain present in the liver in this way (because of the variability in the rate of its absorption from the gastro-intestinal tract), but we anticipated that we could secure qualitative evidence of its fixation after the intravenous injection of massive doses, which were equal to many times the fatal oral dose when administered in pure aqueous solution.

Arnaud (3) reported that ouabain is non-toxic when administered orally, and this statement has been repeated frequently by other investigators, but it is well known that the tolerance of the cat and dog toward pure solutions of ouabain, administered orally, is strictly limited, although, as previously stated, the rate of absorption is quite variable. The oral administration of an amount equal to ten times the fatal vein dose almost invariably gives rise to toxic symptoms, and in these feeding experiments we had administered amounts equal to one hundred times the fatal vein dose.

Massive doses of ouabain were injected intravenously into cats; at death the livers were excised and fed to other cats, some

of which vomited, while others showed no effects. In control experiments large amounts of ouabain were added to meat and liver, which were fed to cats and dogs, but it no instance were we able to kill either of these animals in this way, even when the amounts of ouabain employed were equal to forty times the fatal intravenous dose. Some of the animals showed no perceptible effects, others vomited, but the emesis may have been due to the raw meat or liver.

The livers of cats and dogs were perfused in the effort to determine whether that organ is capable of fixing or destroying the poison. The results of some of these experiments were negative, but in others the evidence of fixation was convincing; however, in no case was the amount fixed comparable to that which leaves the blood within a few minutes after the intravenous injection of a massive dose. We must suppose, therefore, that in the cat and dog the liver has the power of fixing a small amount of ouabain, but that this capacity is far less than that of the liver of the rat.

An abstract of the protocol of one of these experiments will be given and the essentials of the series will be discussed, but it is not convenient to tabulate the results of all of them because of the variety of conditions under which the perfusions were carried out.

A cat, weighing 3.22 kgm., was exsanguinated during the injection of 150 cc. of Locke's solution, 230 cc. of blood and fluid being obtained. This was defibrinated, strained, and to it were added 10 mgm. of ouabain and enough normal saline to make250 cc.

This fluid was perfused about twenty-five times through the portal circulation of the excised liver, with frequent oxygenation, during two hours at a temperature of about 37°C.

The perfused fluid then measured 230 cc., after which the liver was perfused with enough Locke's solution to obtain an additional 265 cc.

The liver, weighing 55 grams, was extracted with 220 cc. of normal saline. The total extract was calculated as 275 cc., the liver being considered as though it were fluid.

Each of the several fluids was then tested on two cats; the following being a summary of the results:

First perfused fluid, 230 cc.	4.50 mgm. ouabain
Second perfused fluid, 265 cc.	0.80 mgm. ouabain
Extract of liver, 275 cc.,	2.75 mgm. ouabain
Total	8.05 mgm. ouabain

It will be seen from the foregoing protocol that the fluid remaining at the end, was less by only 20 cc. than at the beginning, of perfusion, but it contained only about half of the ouabain which had been added, while a larger volume of the second perfused fluid contained about one-sixth as much as the first. It is impossible to say whether the small amount present in this fluid was removed from a loose combination in the vessel walls, or by washing out the residuum of the first perfused fluid which had become mixed, by diffusion, with the fluids of the liver. There is no reason to doubt, however, that the liver had actually fixed a considerable proportion of the ouabain, and it had probably destroyed a smaller amount.

The liver of a dog was perfused for three hours with 1000 cc. of a mixture of defibrinated blood and Locke's solution to which 25 mgm. of ouabain had been added, after which 925 cc. of perfused fluid were obtained, the liver having become swollen. It was then perfused once with 500 cc. of Locke's solution after which the liver was extracted in the usual way. Tests of the several fluids indicated the presence of the following amounts of ouabain:

First perfused fluid, 925 cc.	12.2 mgm.
Second perfused fluid, 500 cc.	1.5 mgm.
Extract of liver	4.2 mgm.
Total	17.9 mgm.
Difference	7.1 mgm.

In another experiment, conducted in an essentially similar way, the perfused fluid contained 80 per cent of the ouabain which had been added, and the test of the extract of the liver failed to show that any of the poison had been fixed by it, but since we neglected to use the "combined ouabain" method in this experiment it is possible that the liver may have contained a small amount.

These experiments show clearly that some of the ouabain was fixed in the liver. Had it been present in loose combination in the walls of the vessels alone it would have been removed during perfusion with the Locke's solution, as indeed, a part of it appears to have been. But it is possible that that which was extracted from the liver required the use of heat in order to render it soluble. It seems much more probable to us, however, in view of the functions of the liver, that the ouabain extracted had been taken up by the cells of that organ and was in process of elimination, either by means of excretion in the bile or by destruction, and it should be stated that in only one or two experiments were we able to recover nearly all of the ouabain added to the perfused fluid.

Several of the perfusion experiments do not call for detailed discussion owing to the lack of sufficient data, such for example, as in the last experiment detailed, or because of failure to secure a satisfactory perfusion.

It may be merely a coincidence that the highest percentages of ouabain recovered in these perfusion experiments, with one exception, correspond closely with the highest percentages recovered from the rat after intravenous injection.

Having found that the removal of the liver, or tying the portal vein, lessens the resistance of the rat to ouabain, we endeavored to learn whether a similar procedure would have a corresponding effect in the cat, although it had been previously shown that no more ouabain was required to kill the dog when it was injected into the portal, that when it was injected into the femoral, vein.

In a series of three experiments, in which the celiac axis, the superior mesenteric artery, and portal vein had been tied previous to the injection of ouabain into the femoral vein, the animals died after the administration of amounts equal to 50, 60, and 70 per cent, respectively, of the average fatal intravenous dose for the normal animal. This would seem to indicate that the liver does exert a considerable degree of protection in the cat, but control experiments showed that after the exclusion of other large vascular areas similar reductions occurred in the amounts of ouabain required to kill the cats. In another experiment the superior mesenteric artery, the aorta (just below the axis), and the

vena cava at the level of the axis, were tied. A dose of 25 mgm. of ouabain was injected into the external jugular vein. The heart stopped in two minutes and forty-one seconds, the animal was exsanguinated, while normal salt solution was being injected into the vena cava, the carotid, and the arch of the aorta, 1040 cc. of fluid being obtained. This was found to contain 9.3 mgm. of ouabain, nearly 16 mgm. having left the blood stream in the restricted area when neither the liver nor the gastro-intestinal tract participated in its removal. This shows that other tissues removed ouabain from the blood, and it suggests the muscle or brain as the seat of its fixation.

While the results just mentioned show that ouabain disappears rapidly from the blood without the participation of the gastro-intestinal tract, except in so far as a small amount of blood reaches it through the collateral circulation, we have removed various abdominal organs and tied the vessels supplying other tissues before injecting the poison, in the further effort to determine how it is removed from the circulation. An abstract of the protocol of this experiment shows that despite the extraordinary degree to which the circulation was restricted—virtually to the heart, lungs, head, and thorax—the poison disappeared from the blood almost as rapidly as in the intact animal.

A cat, weighing 4.46 kgm., was anesthetized, and the gastrointestinal tract from the esophagus to the rectum, the kidneys, spleen, and pancreas were removed. The subclavian arteries and veins, the celiac axis, the superior mesenteric artery, the portal vein, the vena cava, above the bifurcation, and the renal arteries and veins were tied.

The animal received 25 mgm. of ouabain intravenously and the heart stopped after two minutes and thirty-three seconds.

The body was exsanguinated so far as possible by injecting normal saline into the cava and other veins, and into the carotid.

The bloody fluid thus obtained measured 1340 cc., and it was estimated that it contained 100 cc. of blood, or about one-third of the total in the animal.

The tests indicated that about half of the ouabain injected had disappeared from the blood of this restricted area.

Fixation in the brain

It is a curious fact that while numerous investigators have attempted to recover various digitalis principles from the hearts of different animals and to estimate them quantitatively, few have attempted to recover these principles from the central nervous system, although their action on the respiratory and vomiting centers in the medulla is as characteristic as that on the heart, and there is abundant clinical testimony that they also act on the cerebrum. We therefore looked to the central nervous system as the probable seat of fixation of large amounts of ouabain in the cat and dog, especially in view of the fact that the poison disappeared from the blood in the experiment just detailed.

The intravenous injection of an amount of ouabain equal to 65 per cent of the average fatal dose caused cardiac standstill in a dog in which the cerebral circulation was interfered with by previously tying the carotid and vertebral arteries; and a somewhat larger relative amount was required to stop the heart of a decapitated dog (hemorrhage having been arrested) during artificial respiration.

The diminution in the amounts required in these experiments is probably explained by the diminished vascular area, but we perfused the brain of a cat with defibrinated blood to which ouabain had been added in order to determine whether fixation occurs. The results were negative and we then attempted to learn whether the intact brain of the animal fixes the poison after its intravenous injection.

In this experiment the abdominal aorta and vena cava were tied below the level of the renals, and 25 mgm. of ouabain were injected into the vena cava toward the heart; the latter ceased to beat in two minutes. The animal was then exsanguinated during the injection of normal saline, 1200 cc. of bloody fluid being obtained. The tissues of the liver, brain and muscles were then pale and practically bloodless. The brain, including the medulla and cerebellum, was extracted with enough normal saline to make 500 cc., and this and the defibrinated bloody fluid were tested. The tests indicated that the blood contained 9 mgm. of ouabain,

and that about 16 mgm. had left the circulation. The extract of the brain (which did not show a trace of color) contained no ouabain whatever. The test animal received 25 cc. of the extract per kilogram, after which it required a little more than the average fatal dose of ouabain to cause death. A control experiment showed that ouabain could be extracted after its injection into the excised brain and estimated quantitatively.

This failure to recover ouabain from the blood-free brain is not incompatible with the fact that it exerts a typical action on that organ, since mere traces, far too small for us to detect, may be sufficient to exert its peculiar effects, especially those on the respiratory and vomiting centers.

Fixation in muscle

We next investigated the rôle of the voluntary muscles in the removal of ouabain from the blood. A cat received an intravenous injection of 25 mgm. of ouabain, and died in two minutes. The test of the defibrinated blood indicated that about two-thirds of the poison injected had left the circulation. One hundred grams of muscle, taken from the thigh, were extracted and the extract tested on two cats, each of which received an amount of the extract corresponding to 10 grams of muscle for each kilogram of weight, after which it required almost the exact average fatal dose of ouabain to cause death. This result is the more surprising in that the muscle must have contained at least a small amount of blood, hence we must suppose that the extract contained at least traces of the poison. It will be remembered that the muscle of the rat was also found to be free of ouabain after the injection of a large dose, but that may be explained partly by the greater capacity of the liver of the rat to fix the poison.

A practically similar result was obtained in another experiment in which 37.5 mgm. of ouabain were injected intravenously into a cat weighing 3.75 kgm. The cat was bled, but only 75 cc. of the blood, or less than half of the calculated total amount in the body, were obtained. The test indicated that about 22 mgm. of ouabain had left the circulation. An extract of 423 grams of the muscles of the thighs was made and tested, the result indi-

cating the presence of about 2 mgm. of ouabain in the total extract; and since so little blood had been removed, when exsanguination was attempted, we must suppose that all, or nearly all, of the 2 mgm. obtained was present in the blood in the muscles.

The hind limbs of a cat were perfused during seventy minutes with a mixture of defibrinated blood and Locke's solution to which 10 mgm. of ouabain had been added. The test of the perfused fluid indicated that very little, if any, of the ouabain had been removed from the fluid during perfusion. We must conclude, therefore, that the voluntary muscles do not fix more than traces of ouabain, during perfusion, at least.

Fixation in the kidneys

It seemed probable to us that the kidneys excrete at least a part of the ouabain administered, but we have been unable to detect such small amounts as may be eliminated in the urine of the dog because the toxicity of the urine is greater than that of any ouabain which it contains and we have no satisfactory means of separating it from the urine. We attempted to determine whether the poison is excreted in amounts which can be estimated in the urine of the dog during diuresis, induced by hypertonic sodium sulphate solution. The urine then secreted being of very low toxicity in proportion to its volume.

Two experiments were carried out on small dogs, each of which received an intravenous injection of 0.5 mgm. of ouabain. The urines were collected during two hours, and two hours and twenty minutes, respectively, and tested on cats by the "combined ouabain" method. The tests indicated the excretion of about 0.04 mgm. of ouabain in the first dog, and of about half as much in the second, in which diuresis was much less than in the first.

The excised kidney of a cat was perfused once with one part of ouabain in two hundred and fifty thousand parts of Locke's solution, after which the fluid was tested on a cat by the "combined ouabain" method, the test indicating that about 7 per cent of the ouabain had been removed from the perfused fluid. Such minute amounts cannot be estimated accurately, however.

Fixation in the heart and lungs

One seldom thinks of the lungs as fixing poisons of this type and of removing them from the blood, but we wished to secure direct evidence concerning their capacity for fixing ouabain. Previous investigators had shown that the heart does not fix important amounts of ouabain; we therefore employed a method in which the circulation is limited to the heart and lungs in order to determine the rôle of the lungs in its fixation.

The protocol, in brief, of an experiment follows. It indicates that not more than traces of the poison is fixed in the heart or lungs. In another experiment of this type, in which 5 mgm. of ouabain were injected, the test indicated the recovery of 80 per cent of the poison, and it is possible that even that estimate is too low.

A cat, weighing about 4 kgm., was etherized and given artificial respiration. The innominate artery and superior vena cava were connected by a tube and cannulas, after which the aorta was tied just beyond the origin of the innominate, and the inferior vena cava, close to the heart.

Ten mgm. of ouabain were injected into the chamber of the left ventricle; the heart stopped after one minute and forty-five seconds. The heart and lungs were then exsanguinated during the injection of enough normal saline to yield 300 cc. of blood and fluid. The vessels and lungs were cut, in order to exsanguinate the tissues more completely, while enough normal saline was being injected to yield 700 cc. of additional fluid.

The tests of these fluids gave the following results:

First fluid, 300 cc.,	8.00 mgm. ouabain
Second fluid, 700 cc.	1.88 mgm. ouabain
Total	9.88 mgm. ouabain

Having failed to detect more than traces of ouabain in the urine and various bloodless tissues, except the liver, after the intravenous injection of massive doses, we considered an attempt to extract the entire body of the cat (exclusive of the skin and intestinal contents) after the injection of a large dose of the poison,

but we decided that the method which we use would not suffice for its estimation in such dilute solutions as the extracts would yield. We therefore abandoned the plan.

Summary

Ouabain disappears rapidly from the circulation of the cat and dog, less than 50 per cent of a massive intravenous dose being present in the blood at death, which occurs within two to three minutes.

Small amounts of ouabain can be detected in the nearly bloodless liver after the administration of such doses, and after perfusion with diluted, defibrinated blood to which ouabain has been added.

The participation of the liver is not essential for the rapid removal of ouabain from the blood, for it disappears almost as rapidly when the circulation is restricted to certain small areas, but not when it is limited to the heart and lungs.

It is probable that small amounts of ouabain are excreted by the kidneys, but it cannot be detected in the urine of the cat or dog after the administration of sub-lethal doses, except possibly after copious diuresis.

Ouabain cannot be detected in the bloodless voluntary muscle or brain after the intravenous injection of massive doses, or in those structures after perfusion with it.

We can offer no other explanation of the rapid disappearance of large amounts from the blood than that the normal liver decomposes the poison more rapidly than the perfused liver, or, that the ouabain is widely distributed in various tissues of the body.

If that part of the ouabain which leaves the blood before death were distributed nearly uniformly throughout the body, the amount present in any one tissue would be insufficient to permit of its extraction and estimation (owing to its dilution in the extract) with a sufficient degree of precision to permit of our determining whether it was present in the blood of the tissues or in the tissues themselves. Our inability to detect such amounts

in extracts of the tissues will be understood when it is remembered that the fatal intravenous dose of ouabain for the cat is only equal to one-ten-millionth of the body weight.

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Some Observations on the Pharmacology of a Digitalis Body

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SOME OBSERVATIONS ON THE PHARMACOLOGY OF A DIGITALIS BODY*

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The investigation with which this paper deals was started with a view to preparing a digitalis principle in a form suitable for intravenous administration following numerous inquiries concerning such a preparation. There seems, however, to be a more imperative need of one which is absorbed from the gastro-intestinal tract with greater uniformity than are the tinctures or other preparations made from digitalis obtained from a variety of sources and including wild grown and cultivated American, English and German leaves, partly from the first year's growth, partly from the second, and dried in a variety of ways.

The need of such a readily absorbable preparation has been called to my attention by the complaints of clinicians who were unable to secure the typical effects of digitalis even with large doses of the tincture in use in the hospitals with which they are associated. Several of these tinctures were submitted for examination, and in every case the biologic test showed that they were of a fair degree of activity.

Certain of the digitalis principles are readily absorbable from the gastro-intestinal tract of man, as well as that of animals, while others are absorbed much less readily, and it seems probable that the failures just mentioned arose from the fact that the preparations contained relatively large proportions of the less readily absorbable active principles.

A preliminary report [1] of a method of separating the more readily absorbable principles from those which are less readily absorbable was made about a year ago to the American Pharmaceutical Association. Since then modifications of that method have

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

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been employed in the effort to separate the more absorbable fraction in pure form and to estimate the amount in the leaf or tincture quantitatively. The substance obtained by the several modifications of the method is essentially the same, though it differs somewhat in purity. The simplest process for obtaining it consists in placing the wetted ground digitalis on a filter paper resting in water and supported by a wire frame, with a layer of chloroform beneath the water. The active principles of the leaf pass into the water by circulatory solution, and the chloroform takes up the more absorbable fraction, leaving the less absorbable in the water. The chloroform is then evaporated spontaneously, no heat or chemicals being used at any stage of the operation.

There is so much uncertainty regarding the chemistry of digitalis that any attempt at a comprehensive discussion of the subject would be out of place here; but certain salient points of resemblance between this chloroform-soluble substance and digitoxin, and certain dissimilarities may be pointed out. It is now generally accepted that digitalis contains digitoxin, digitalin, and one or two other active principles. Digitoxin is the only one of these that is soluble in chloroform, while all are readily soluble in alcohol, and none except the ill-defined digitalein is easily soluble in water. Nevertheless, water extracts all of the active principles of digitalis almost as readily as alcohol, a circumstance that affords evidence that these principles exist in the leaf in combination, and not in the free state as we know them.

The chloroform-soluble substance resembles digitoxin to a certain degree, both chemically and pharmacologically, and it seems probable that it contains combined digitoxin, though not in its original combination, because when once it is separated the substance is much less soluble in water than before; and furthermore, when it is separated there remains another substance which is no longer soluble in water, and which is evidently thrown out of solution by the removal of the chloroform-soluble fraction.

The chloroform-soluble substance is resinous in character, brittle when cold, but softening with heat; it has a fragrant odor and an intensely bitter taste; it is soluble in chloroform and in alcohol in all propor-

tions, and when water is added to a not too concentrated alcoholic solution the mixture remains clear or slightly fluorescent, though the substance is only slightly soluble in water; it is insoluble in petroleum benzin and in anhydrous ether, but ether dissolves a part of some specimens, leaving a part which is nearly indistinguishable in appearance and in pharmacologic action from the more soluble portion. If a fragment is dissolved in glacial acetid acid, a trace of ferric chlorid added and the solution allowed to flow over an equal volume of sulphuric acid contained in a test tube, without mixing, a dark zone appears at the line of contact, and the acetic acid gradually assumes a green, and then a blue, color, gradually fading to a dirty appearance, the reaction being identical with that given by digitoxin except that in addition the chloroform-soluble substance gives a ruby red color in the upper part of the sulphuric acid.

The behavior of digitoxin following its subcutaneous injection in the rat is peculiar, in that the animal, which excretes certain other digitalis bodies (ouabain and amorphous strophanthin) with extraordinary rapidity, shows a peculiar train of symptoms in which clonic convulsions are prominent, lasting for a period of about a week with just fatal doses. The symptoms following the subcutaneous injection of a just fatal dose of the chloroform-soluble substance are indistinguishable from those following similarly active doses of digitoxin. The rabbit eliminates a nearly fatal dose of digitoxin in a period of two or three hours in marked contrast to the cat and rat. It is significant that the chloroform-soluble substance is also eliminated by the rabbit at almost exactly the same rate as digitoxin. Such striking similarities of behavior in these two animals can hardly be a mere coincidence. The accompanying table gives certain points of resemblance between this chloroform-soluble substance and digitoxin.

One who has never worked with digitalis or other drug of similar complexity can have no adequate comprehension of the extraordinary variety of compounds which are obtained with slight variations in the method of extraction, while the changes in solubility of the active principles in different menstruums, resulting from slight differences in the impurities present, are confusing at times.

In marked contrast to the ease with which these combinations are broken down or altered is the stability of the most active of the principles which resists long continued boiling in weakly acid or neutral solution, and which I have obtained from a specimen of the fluid extract which had stood for nearly forty years. The active substance is associated with a saponin-like material, the greater part of which is separated easily but the last traces of which are removed only with some difficulty. It is difficult to remove traces of chlorophyl with which it is sometimes contaminated.

COMPARISON OF THE CHLOROFORM-SOLUBLE FRACTION OF DIGITALIS WITH DIGITOXIN

	Digitoxin	Chloroform-Soluble Substance
	White crystalline powder	Greenish; resinous
	Melts at 240 C.	Melts gradually at about 100 C.
	Odorless	Fragrant
	Intensely bitter	Intensely bitter
	Solubilities	
Alcohol	Easily soluble	Very soluble
Chloroform	Very soluble	Very soluble
Ether	Insoluble	Insoluble
Petroleum benzin	Insoluble	Insoluble
Water	Insoluble	Nearly insoluble
Color reaction	Positive	Positive (with ruby red)
Action in rat	Peculiar, hyperexcitability	Indistinguishable from that of digitoxin
Elimination, rabbit	Extraordinarily rapid	Extraordinarily rapid
	Fatal dose per kilogram (Approximate)	
Cat, vein	0.35 to 0.5 mg.	1.0 mg.
Rabbit, vein	1.0 mg.	2.5 mg.
Frog, lymph sac	8.5 mg.	10. mg.

It was earnestly hoped that a method might be perfected for the quantitative determination of the chloroform-soluble fraction in digitalis leaves or the tincture in order that the dosage might be determined with reference to the content of this readily absorbable fraction, but the effort has not been successful. It appears to be merely a question of overcoming certain difficulties, and it is hoped that a satisfactory method of doing this may be devised.

The chloroform-soluble, or more absorbable, fraction and the aqueous residue, containing the less absorbable digitalis principles, are capable of exerting

the typical digitalis action as shown on the frog, in which they induce ventricular systolic standstill, preceded by slowing, irregularity and dissociation of auricles and ventricle, and also by an electrocardiogram (for which I am indebted to Dr. R. Edwin Morris of the University of Minnesota) which shows a typical lengthening of the P-R interval with small doses and complete dissociation of the auricles and ventricles with large doses, together with a gradual flattening and inversion of the T wave in the cat. It also causes emesis through stimulation of the vomiting center in the cat and dog.

The relative absorbability of the two fractions (the chloroform-soluble, and the chloroform-insoluble) was studied in the following manner: They were assayed biologically by the cat method, which determines the amount required per kilogram of weight when injected intravenously to kill the animal, such an amount being called for convenience a cat unit. The fractions were then administered to cats through a stomach tube in doses varying from one to four cat-units per kilogram of weight, and the symptoms induced were observed. The results were not uniform, but the chloroform-soluble fraction proved to be much the more readily absorbable. The chloroform-soluble fraction is relatively less actively emetic than the residue, apparently, the intravenous injection of about 30 per cent. of the average fatal dose of the residue causing emesis in cats, while about 40 per cent. of the average fatal dose of the chloroform-soluble fraction is required; but the oral doses of as little as one cat unit per kilogram of weight of this fraction usually caused emesis in a short time, whereas oral doses of three and four cat units per kilogram of the aqueous residue sometimes failed to produce any perceptible symptoms. On the other hand, the absorption of the aqueous residue was more irregular than that of the chloroform-soluble fraction, and of two animals that received one, and two and a half, cat units per kilogram, respectively, of the same specimen of the residue the one receiving the smaller dose was nauseated and depressed, while the other showed no perceptible effects. The persistence of action in each case was determined on the following day by the differential test

with ouabain (difference between the average fatal dose for a normal animal and that actually required for these cats); and while a single test is not conclusive, the results supported the evidence of absorption just mentioned; the cat which received the larger dose of the aqueous residue required much less of the ouabain than the one which had had the smaller dose. It may be remarked parenthetically, that we have found that the absorbability of several of the digitalis bodies from the gastro-intestinal tract of the cat closely parallels that observed in man.

An effort was made to estimate the relative amounts of the two fractions in a tincture and in a fluidextract which had been sent for examination because of their failure to induce the typical digitalis actions in man when used clinically in large amounts. Though it is impossible to separate these fractions quantitatively, it is at least significant that both of these preparations yielded very low percentages of the more absorbable fraction, and there is little reason to doubt that this fact explains why they proved ineffective in man.

We are especially concerned with the uniformity of action of the tinctures which we use by any given method of administration, and not with the presence in them of a variety of substances, including acids, volatile oils, saponins, resins, chlorophyl, sugar and coloring matter not to mention different active principles of unknown and varying degrees of activity; but since different tinctures vary in their absorbability, and therefore in their activity when administered orally, even though they may be of uniform activity when tested biologically, there is need of a preparation of as nearly uniform absorbability and activity as can be obtained. I wish to lay special stress on the fact that if one could always use the same tincture of digitalis, one would encounter little difficulty with its use after he had learned the dose, and he would probably never have an occasion to use such a preparation as the one now under consideration.

It is with this fact in mind that I present this paper dealing with a substance which I believe to be the most active principle of digitalis in a form more closely resembling that in which it exists in the leaf than any that has been prepared hitherto.

A digitalis body [2] designed for intramuscular or intravenous injection must meet the following requirements: It must be soluble in water, or at least in a weakly alcoholic menstruum; it must be of uniform activity; it must exert a minimum of undesired side actions; it must not deteriorate rapidly, and it must not irritate the veins or tissues into which it is injected.

It is commonly assumed that the highest degree of purity is essential for a preparation which is injected intravenously; but while such a degree of purity is highly desirable, it is not an essential, provided the impurities present are not injurious. Tinctures of digitalis from different sources vary widely in their composition; nevertheless they have been injected intravenously with apparently satisfactory results. If clinicians have felt impelled to use the tincture in this way despite its disadvantages, it is obvious that any true digitalis body which possesses the essentials already mentioned must be preferable to the tincture.

The chloroform-soluble substance meets the first of these requirements in that it is readily soluble in alcohol and is then readily miscible with water, the solution thus formed being fairly stable; the uniformity of activity is assured by the biologic assay; it is about as actively emetic as tincture of digitalis, certainly not materially more so; so far as can be determined it does not deteriorate more rapidly than tincture of digitalis; the veins of cats were examined some days after toxic doses had been injected into them and found to be free from evidences of irritation, and numerous doses were injected into one of the ear veins of a rabbit during a period of several weeks. No evidences of pain could be elicited following the intramuscular injection into cats, but the following experiment on man is worthy of notice: An eighth of a milligram of the active substance, equal to an eighth of a cat unit, in 2,000 parts of physiologic sodium chlorid solution containing about 10 per cent. of alcohol, was injected into the deltoid (of the author). After about ten minutes there was a slight burning

2. While the terms "digitalis body" and "principle" are used in this paper, it is to be understood distinctly that the substance under consideration is not an alkaloid or glucosid in pure form. The substance probably consists of digitoxin mainly, and it is probably in combination much as it exists in the leaf, although this is only a surmise.

pain lasting for about twenty minutes. When this had become imperceptible during rest, a sudden violent movement of the arm was made with the purpose of determining whether pain could be elicited. There was a sharp burning pain lasting a minute or two, but this could not be elicited on repeating the movement. There was a slight sense of soreness during the following twenty-four hours when the arm was used, but this was not sufficient to attract the notice. The deltoid is more sensitive than the gluteus, but the dose was much smaller than that required for the full therapeutic effects; hence it is impossible to state whether the pain of the intramuscular injection of a therapeutic dose into the gluteal or lumbar region would be too painful or not.

From the foregoing it seems reasonable to conclude that the weakly alcoholic solution is suitable for intravenous, and possibly for intramuscular, injection in man, but that its ready absorbability following the oral administration renders other modes of administration superfluous in nearly all cases.

SUMMARY

Water exhausts ground digitalis leaves, and from the infusion thus prepared chloroform removes a principle which resembles digitoxin somewhat, both chemically and pharmacologically, and which is more readily absorbable from the gastro-intestinal tract of the cat than is the residue remaining in the infusion.

The chloroform-soluble substance is soluble in alcohol, and the alcoholic solution is miscible with water without precipitation, the weakly alcoholic solution undergoing little change in a period of about a year during which it has been under observation.

It is a true digitalis body, in that it exerts the typical cardiac action of the latter. It is not more actively emetic than is digitalis in proportion to its therapeutic effects.

It is believed that it is suitable for intravenous, and probably for intramuscular, injection, in the relatively few cases in which such methods of administration may be necessary.

It is hoped that a way may be found to estimate the amount of this readily absorbable fraction in digitalis so that the powdered drug, the tincture or the infusion

may be used instead of this chloroform-soluble fraction, which is naturally more expensive than the official preparations.

Further pharmacologic studies of the substance are in progress in the hope that the preparation may be made official, should the clinical studies show that it possesses any advantages over the official tincture.

Reprinted from The Journal of the American Medical Association
Aug. 14, 1920, Vol. 75, pp. 460-463

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THE ABSORPTION OF A DIGITALIS BODY

PRELIMINARY OBSERVATIONS *

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NEW YORK

The greatest diversity of opinion exists relative to the rate and completeness of absorption of digitalis from the human alimentary canal, and very few accurate observations have been made in this important field. In a study of the dosage of digitalis [1] published five years ago, it was shown that the tincture and the infusion, and commercial crystalline digitoxin, were generally absorbed both rapidly and fairly uniformly from the gastro-intestinal tract of man when adequate doses were administered. The precise rate of absorption was not determined, but the fact was brought out that the absorption of a single dose of digitalis was apparently complete within about six hours after its ingestion. It was also proved that full digitalization could be brought about by oral administration within from ten to twenty hours from the beginning of treatment. These observations have been confirmed by White and Morris,[2] and by Robinson.[3]

Robinson showed that the initial effects of digitalis appear in from two to five hours after the oral administration of a single large dose of the tincture, and in an unpublished report on the rapidity of absorption of digitalis from the digestive tract of man, H. E. B. Pardee concluded from his observations that the absorption of a single dose of the tincture is prac-

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* Read before the Section on Pharmacology and Therapeutics at the Seventy-First Annual Session of the American Medical Association, New Orleans, April, 1920.

1. Eggleston, Cary: Digitalis Dosage, Arch. Int. Med. **16**: 1 (July) 1915.

2. White, S. M., and Morris, R. E.: The Eggleston Method of Administering Digitalis, Arch. Int. Med. **21**: 740 (June) 1918.

3. Robinson, G. C.: The Rapidity and Persistence of the Action of Digitalis on Hearts Showing Auricular Fibrillation, Am. J. M. Sc. **159**: 121 (Jan.) 1920.

tically complete in about six hours, while a marked degree of absorption is almost always demonstrable in from two to four hours.

These and other observations indicate that, as a general rule, digitalis is rapidly and fairly well absorbed from the human alimentary tract; and the fact that an average oral dose could be established shows that absorption generally takes place with a considerable degree of uniformity. This is borne out by an analysis of the full therapeutic doses recorded in sixty-six cases representing all forms of heart disease, collected from the papers of Pardee [4] and myself, in which only two, or 3 per cent., of the patients showed excessive variation from the average, and in both of these the administration was continued over a considerable period of time. One of these patients required nearly two and one half, the other three, times the average dose.

Admitting the occasional occurrence of a patient who proves unusually tolerant to digitalis through one or another unknown cause, there remains the much more important occurrence of specimens of digitalis which are for some inherent reason poorly absorbed, irrespective of the patient. Thus, Dr. B. S. Oppenheimer [5] records three patients who consumed enormous amounts of a given sample of digitalis without evidences of either therapeutic or toxic action. One of his patients took 540 minims of the tincture and 21 ounces of the infusion (same digitalis) in twenty-three days, equivalent to 134 c.c. of the tincture. A second took 3,090 minims (193 c.c.) of the tincture in thirty-eight days, and the third took 3,735 minims (233 c.c.) of the tincture (or its equivalent in infusion) in forty-three days. These amounts are from five to nine times the maximum average dose required for a sample that is absorbed as well as the average. Even when allowances are made for the long periods over which administration was continued, it is evident that the particular lot of digitalis employed was very poorly absorbed. Similar observations are reported by Wedd,[6] who records five patients who took of a given sample from

4. Pardee, H. E. B.: Notes on Digitalis Medication, J. A. M. A. **73**: 1822 (Dec. 13) 1919.

5. Oppenheimer, B. S.: Personal communication to the author.

6. Wedd, A. M.: Observations on the Clinical Pharmacology of Digitalis, Bull. Johns Hopkins Hosp. **30**: 131 (May) 1919.

four to six times the average total dose with no toxic symptoms, and with little or no therapeutic effect. Other instances have come to our attention, but we have insufficient data to warrant their inclusion; nor would anything be added by rehearsing them, since the foregoing are sufficient to indicate how poorly some samples of digitalis are absorbed.

The observations reported by Hatcher,[7] in which he shows that digitalis can readily be separated into two distinct fractions by extraction of its aqueous solution with chloroform, and his observations on the marked differences in the absorbability of the two fractions from the alimentary canal of the cat, opened the way for the clinical study of the absorption of digitalis from a new angle. This study, though begun some months ago, has not progressed as rapidly as was hoped, partly owing to the crippling of our service by the influenza epidemic. I regret, therefore, that I can at present make only a preliminary report, which, however, shows results in close harmony with those obtained by my colleague, Dr. Hatcher, on animals.

In order first to compare the absorption of each of the two fractions with the results previously obtained with various samples of tincture of digitalis made from high grade leaves, the fractions were supplied to me in the form of artificial tinctures in a menstruum of 60 per cent. alcohol. All the samples were prepared so that 1 c.c. represented one cat unit in activity. These were administered to patients and the whole procedure was made to parallel as closely as possible the original plan followed in the determination of the average total human dose of the tinctures. In this way the results could be compared directly with those secured from the use of the official tinctures.

Twenty-three complete courses of administration were carried out in twenty-one patients, using four different samples of the chloroform-soluble, and one of the chloroform-insoluble, fractions. Each of the chloroform-soluble fractions, 8-3-19, 9-23-19 and 2-3-20, was obtained from a different lot of digitalis leaves, while the fraction 3-18-20 was made by mixing the chloroform-soluble extracts obtained from a large number of different samples of digitalis. The chloro-

7. Hatcher, R. A.: Some Observations on the Pharmacology of a Digitalis Body, J. A. M. A., this issue, p. 460.

form-insoluble fraction 9-23-19 was obtained from the same leaf as the chloroform-soluble fraction of the same number.

The determination of the average total therapeutic dose of each of the chloroform-soluble extracts was the first problem attacked, since its solution would provide the necessary data for the investigation of other problems. The full therapeutic dose of one sample of the chloroform-insoluble fraction was also determined approximately. The results of this phase of the investigation are set forth in the accompanying table, in which the corresponding figures for the tincture of

AVERAGE FULL THERAPEUTIC DOSES AND PERCENTAGE VARIATIONS FROM THE AVERAGES, FOR EACH FRACTION, FOR ALL CHLOROFORM-SOLUBLE FRACTIONS COMBINED, FOR TINCTURES OF DIGITALIS, AND FOR CRYSTALLINE DIGITOXIN

Preparation	Full Therapeutic Dose, Average	Number of Cases	Per Cent. Above	Per Cent. Below
Chloroform-soluble fractions:				
8-3-19	0.153	3	11	20
9-23-19	0.161	6	26	28
2-3-20	0.157	5	20	18
3-18-20	0.182	1	..	..
All chloroform-soluble fractions	0.159	15	28	26
Chloroform-insoluble fractions:				
9-23-19	0.640	4	19	30
Tinctures	0.148	25	97	66
Digitoxin*	0.066	15	66	43

* One very tolerant patient is included in this group; if omitted, the average for the fourteen remaining cases is 0.063, with maximum variations of 35 per cent. above and 40 per cent. below.

digitalis (seven different specimens) and for crystalline digitoxin are included for comparison.

Reference to the table shows that the average full therapeutic dose of each of the first three chloroform-soluble extracts studied is approximately the same. The average total dose, calculated from fifteen instances in which one or the other of the four different samples of extract was used, is but slightly higher than the corresponding average for tinctures, as determined from twenty-five cases. The activity of the chloroform-soluble fraction for man, therefore, is not materially different from that of the average high grade tincture of digitalis, when the comparison is made on the basis of the biologic activity of each as determined by the cat method of assay.

The chloroform-soluble extract contains an average of about 1 mg. of solid per cat unit, while the amount of solid per cat unit of the official tincture of digitalis varies widely—between 28 and 44 mg. in three samples tested. These figures give some idea of the degree of purification of the extract. This is further shown by the fact that commercial crystalline digitoxin has an activity of from 0.35 to 0.4 mg. per cat unit, or only about three times that of the chloroform-soluble extract. Dr. Hatcher has shown that the extract gives the typical color reaction for digitoxin, and that it presents many close resemblances to digitoxin pharmacologically. In spite of these close similarities, he has shown that the chloroform-soluble extract of digitalis is not the same as crystalline digitoxin, but that it is probably a somewhat altered combination of digitoxin. It is of interest in this connection, therefore, to compare its behavior in man with that of crystalline digitoxin. The table shows that for man the activity of digitoxin is about two and one-half times that of the purified extract, when the comparison is made in terms of the biologic activity of each as determined by the cat method. When, however, the activity for man is compared in terms of weight, it is found that crystalline digitoxin is about seven times as active as the purified extract. These comparisons make it certain that the chloroform-soluble extract is not the same substance as crystalline digitoxin, and confirm the results of the laboratory investigations previously cited.

Turning now to the question of the absorption of the chloroform-soluble extract, it is seen from the table that the range of variation of dose from the average is much smaller for each of the three fractions investigated, and for all combined, than it is for representative good tinctures of digitalis. Thus, for the chloroform-soluble fraction the greatest divergence from the average dose amounts to only 28 per cent. of the average; that is, one patient of the fifteen developed full therapeutic effects only after he had received 28 per cent. more than the average dose required. This contrasts strikingly with the variation noted among a variety of tinctures obtained from different sources, which rises to 97 per cent. above, and falls to 66 per cent. below, the average. Further, while no variation greater than 28 per cent. of the average dose occurred

in a series of fifteen patients receiving the chloroform-soluble fractions, six patients receiving the various tinctures of digitalis showed variations greater than 40 per cent. of the average dose, in a series of twenty-five.

These facts show that the chloroform-soluble extract of digitalis is absorbed from the human alimentary tract much more uniformly than are high grade tinctures of digitalis obtained from different sources, and it should be recalled that some samples of the official tincture obtained from reliable sources are so poorly absorbed that amounts from four to nine times the average full therapeutic dose fail to produce any demonstrable effects, or at most produce only slight and insufficient effects. The fact that the chloroform-soluble extract is absorbed from the human digestive tract with much greater uniformity than is the tincture of digitalis is alone sufficient to render it a valuable advance in the pharmacy of digitalis, even if it should prove to have no other advantages over the cruder preparations. Although at present no definite statements can be made on the basis of clinical investigation, it seems probable that the chloroform-soluble extract will prove to have other advantages, especially on account of its relative freedom from impurities and its ready miscibility with water, so that it should be available for intravenous injection.[8]

Before leaving the subject of the irregularity of the absorbability of commercial tinctures of digitalis which are derived from different sources, I want to call attention to the fact that the biologic activity of the tincture is not necessarily related to its absorbability from the human alimentary tract. This is well illustrated by the fact that the tinctures which Oppenheimer and Wedd found to be so poorly absorbed were both of approximately average biologic activity, and both were considerably more active than some of the tinctures which I found to be absorbed fairly well and relatively uniformly. It seems certain that this fact will necessitate a radical change in the method of assay of digitalis for human use, and Dr. Hatcher is now approaching the problem of determining both the activity and the absorbability of digitalis as a means of reaching a more accurate estimate of its therapeutic value.

8. The chloroform-soluble extract has since been administered intravenously to one patient with satisfactory results and without untoward effects.

The precise rate of absorption of the chloroform-soluble extract has not yet been determined for man, but the evidence so far obtained indicates that it is absorbed at least as rapidly as the most readily absorbed tinctures, and I can cite one case which was examined at least every hour and in which the maximal therapeutic action had developed within two hours and a half after a single dose amounting to about 0.112 cat unit per pound (15 c.c. total). The persistence of action of this extract has also not yet been determined for man, but again the evidence so far obtained indicates that it is equal to the best digitalis in this respect.

The chloroform-soluble extract has been supplied to several others for the study of its behavior in man, and we have reports from two who have used it in several cases. Dr. B. S. Oppenheimer of New York states that "the purified tincture of digitalis has proved uniformly satisfactory." Dr. R. W. Scott of Cleveland has administered it in eight carefully controlled cases and reports that "(1) digitalis action occurred in from one to two hours after 10 c.c. [single dose] was given by mouth. The certainty with which one could predict a definite improvement in the circulation in from eight to ten hours was indeed striking. . . . (3) Thus far the therapeutic effects have been fairly uniform as indicated by the level to which the heart beat was slowed, with resulting circulatory improvement."

I want to mention the results of the administration of the chloroform-insoluble extract to five patients, two of whom also received the chloroform-soluble extract from the same leaf on another occasion. The average full therapeutic dose of the chloroform-insoluble fraction is about four times that of the chloroform-soluble, in terms of biologic activity, indicating that in man as well as in the cat it is absorbed very poorly following oral administration. Other studies on its behavior in man have not been made as yet, owing to the obvious lack of value of the preparation.

SUMMARY

1. The fairly rapid and complete absorption of digitalis from the human alimentary canal is shown for a number of different high grade tinctures of digitalis.

2. Contrasted with this is the very poor absorption of certain tinctures, of which from four to nine times the average therapeutic dose failed to produce therapeutic effects.

3. The marked uniformity of absorption of the chloroform-soluble extract of digitalis prepared from a variety of different leaves is shown and contrasts sharply with the variations noted in the cases of tinctures from a variety of sources.

4. The chloroform-soluble extract is shown to be absorbed at least as rapidly as the best tincture of digitalis, and its persistence of action is apparently of the same order as that of digitalis of the best grade.

5. The observations indicate that for oral administration the chloroform-soluble extract is not superior to a well absorbed tincture of digitalis, but it is far superior to tinctures which are derived from a variety of sources, the absorption of which shows very marked variations when the individual specimens are compared.

6. The chloroform-insoluble extract is very poorly absorbed from the human alimentary tract, as well as from that of the cat.

412 West End Avenue.

Reprinted from The Journal of the American Medical Association
Aug. 14, 1920, Vol. 75, pp. 463-465

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Reprinted from The Journal of Pharmacology and Experimental Therapeutics
Vol. XIII, No. 5, August, 1919

A FURTHER CONTRIBUTION TO THE PHARMACOLOGY OF THE LOCAL ANESTHETICS[1]

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Received for publication June 3, 1919

The search for substitutes for cocain has led to the introduction into the materia medica of a large number of compounds possessing the property of causing local anesthesia, and for each of these the claim has been made that it is less toxic than cocain. These compounds belong to several general chemical groups. As we shall show later, not only do these compounds of somewhat diverse chemical nature possess the common property of producing local anesthesia, but also they resemble one another very closely in all their more important pharmacologic actions, such differences as are shown being chiefly quantitative. That they are all capable of producing severe, or even fatal, acute poisoning in man, with symptoms closely analogous to those produced by cocain, will also be seen in the brief review of the more important literature which we give before passing to the presentation of our experiments.

REVIEW OF THE LITERATURE

The occurrence and symptoms of acute cocain poisoning are so well known that it is unnecessary to do more than refer to one or two articles. Mattison (1) presents in abstract a large number of acute fatal and non-fatal cases of cocain poisoning; and a good bibliography of cocain poisoning is to be found in a

[1] Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

paper by Wildenrath (2). In a previous paper we (3) cited the fact that death has been recorded following such small doses of cocain as 16, 40 and 60 mgm., respectively, while 1.25 grams, injected subcutaneously, have been survived. The typical symptoms and course of acute, non-fatal intoxication are described by Lodge (4). The patient, a man forty years old and in good health, received a subcutaneous injection of 20 minims of a 2 per cent solution of cocain—about 13 mgm. of the drug. In two to three minutes the patient became nervous with heightened reflexes, rapid pulse, deepened respiration, vertigo, and flickering before his eyes. Nausea developed in about ten minutes, followed by vomiting. At the end of about an hour after the injection the toxic symptoms were at their height, when the following were noted:

> Pulse 120, respirations deepened, the inspiratory time probably increased to four times the length of the expiratory time; reflexes greatly increased, clonic spasms of all the muscles of the limbs, arms and inferior maxillary . . . . pupils markedly dilated . . . . muscles of the lower jaw were in tonic contraction.

The patient recovered in about two hours after the injection, but he did not sleep well that night, and had a severe headache the next day. This case illustrates the promptness of beginning absorption and the rather prolonged action of the cocain—both facts of importance, as will be shown subsequently.

The literature of tropacocain is comparatively meagre, and we have not found any record of fatal intoxication in man from the use of this drug. We venture to express it as our belief, however, that this drug is little, if any, less toxic for man than is cocain, though it is possible that man may not show the same idiosyncrasy toward it as he does toward cocain.

Marcinowski (5), in an extensive paper on the clinical uses of beta-eucain, comments on its low toxicity for man. He says that cocain is 3.75 times as toxic for animals as beta-eucain, but he does not mention the species, the mode of administration, or the concentration of the solutions, hence his figures are virtually worthless. He gives a bibliography of 202 papers, chiefly clin-

ical, and he goes so far as to state that where beta-eucain can be substituted for cocain it is little short of criminal negligence not to do so. He also makes the direct statement that in man general intoxication has been seen only after the use of beta-eucain in the spinal canal.

Kraus (6) records a case of severe acute poisoning from the injection into the urethra of a healthy man of 0.2 gram of beta-eucain in 2 per cent solution. The day preceding, the same dose had been similarly injected for the incision of a stricture, and had produced no symptoms whatever. He believes that the acute intoxication resulted from the rapid absorption of the drug through the recent wound.

Way (7) also records a case of serious intoxication from the infiltration about the genitals for circumcision of 0.12 gram of beta-eucain in a solution containing a few drops of epinephrin. In both this and the preceding case the symptoms appeared promptly after the injection, and in both clonic or tetanic convulsions were noted. Recovery was apparently more rapid in Way's patient than is seen after cocain, while Kraus' patient still had some symptoms after the lapse of nearly two hours.

The more important literature of intoxication by stovain has been reviewed in a paper by Smith and Hatcher (8), to which the reader is referred. It is well known that many cases of serious, or fatal, intoxication have resulted from the use of this drug, especially for intraspinal anesthesia.

Alypin was introduced into the materia medica by Impens and Hofmann with claims of superiority over cocain and the cocain substitutes, and Impens (9) presents an extensive pharmacologic study of its actions and toxicity, in which he states that for cats and dogs alypin is only half as toxic as cocain by subcutaneous injection. He concludes from his experiments that the danger from alypin in man must also be much less than that of cocain. He, however, shows clearly that the toxic actions of alypin and cocain are essentially the same qualitatively in the lower animals, although he contends that there is less tendency to variations in the responses of different animals to a given dose of alypin than to cocain.

That severe intoxication does occur with alypin is seen by the following references to recorded cases. Garasch (10) records two cases of severe poisoning resulting from the intraurethral instillation of 5 cc. of a 5 per cent solution, and 5 cc. of a 2 per cent solution, respectively (0.25 and 0.1 gram, respectively), in two young men for the purpose of instrumentation. The symptoms began in one and a half to two minutes, rapidly reached their maximum, and both patients had fairly completely recovered within about twenty minutes. In both instances the heart and respiration had stopped, or nearly stopped, and convulsions were repeated. The author says that these are the only cases of severe intoxication from alypin in his total of 1453 cases of its use in genito-urinary surgery. He also records the injection into the bladder of an old man with carcinoma of that viscus of 15 cc. of a 10 per cent solution (1.5 grams) of alypin without the production of any symptoms.[2]

Ritter (11) records an instance of acute intoxication resulting in death, following the infiltration into the region of the thyroid gland of about 1 gram of alypin in 2 per cent solution. Although the initial symptoms were typical of acute alypin poisoning, it is not certain that death was wholly due to that drug, as the patient was suffering from obstructed breathing due to a goiter, and had also received morphin and adalin before the infiltration.

Other cases of severe, or fatal, intoxication have been reported; thus Proskauer (12) records sudden death after injection into the urethra of 20 cc. of 2 per cent solution of alypin (0.4 gram) following injury to the mucosa by attempted passage of a cystoscope; and Schroeder (13) records two non-fatal cases following intranasal infiltration of 13 cc. and 18 cc., respectively, of a 2 per cent solution of alypin (0.26 and 0.36 gram), followed by application by spray and tampons of a 15 per cent solution in small amounts. Both of the patients had previously received 0.6 mgm. of scopolamin. In both patients the symptoms of intoxication began in less than ten minutes after the

[2] Translated by Mr. Solomon Krell, technical assistant.

injection of the alypin, and both recovered promptly after artificial respiration and the administration of cardiac stimulants. Jacobs (14) records two non-fatal cases following the intraurethral instillation of 2 per cent alypin solution, both patients having sustained injury to the urethra by previous effort to pass an instrument. Each of these patients received a dose of about 0.16 gram of alypin, and although both had violent clonic convulsions and stopped breathing, recovery was very rapid following artificial respiration.

We have not found any references to severe acute poisoning by either holocain or nirvanin, but this is probably due to the fact that neither of these drugs has been extensively employed in clinical practice. Certainly it is not due to their slighter toxicity.

The question of accidental poisoning by procain (novocain) has been reviewed in our earlier paper and does not require further mention, except that we wish to emphasize the fact that in the non-fatal cases recovery from the threatening symptoms has generally been rapid. Following the publication of that paper a plea for the reporting of all cases of accidents from the use of local anesthetics was published broadcast in the medical, dental and pharmaceutical journals, signed by Torald Sollmann, chairman of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry, and by Robert A. Hatcher. Although more than a year has elapsed since that plea must have reached every physician, dentist and pharmacist who reads his journals, only four cases of procain, and five of cocain, poisoning have come to our attention, all privately reported. In but two of these cases were the details given sufficient to warrant their mention. One was a case of severe intoxication in a woman thirty years old and on the verge of a nervous breakdown. Forty minims of 2 per cent solution of procain (about 50 mgm.), containing 1-50,000 epinephrin, were injected into the mandibular foramen and less than eight minutes later the patient developed clonic convulsions and slowing of the pulse. Camphorated oil and aromatic spirit of ammonia were administered and recovery was complete in less than two hours. The second case resulted

from the massage into the nose of about 65 mgm. of cocain in a solution containing 10 drops of 1-1000 epinephrin. The patient promptly had three seizures of clonic convulsions with great weakening of the heart but recovered in half an hour, aromatic spirit of ammonia having been employed as a stimulant.

Two facts seem to stand out clearly from the preceding review:

1. That moderate doses of cocain and its substitutes are capable of producing severe, or fatal, acute intoxication in man under a variety of conditions, both with respect to the patient and to the mode of application of the drug.

2. That the rapidity of absorption of the drug into the blood stream seems to be the factor of greatest importance in determining the occurrence of acute intoxication, especially in the case of the several substitutes for cocain.

It will be admitted by all that it would be of the greatest value to determine accurately the several factors which lead to the production of such acute intoxications; their relative importance; their relative frequency of occurrence; and the methods of anticipating them or of avoiding them. But, while animal experiments may throw some light on these problems, their accurate solution can come only from the collection and careful study of clinical accidents. It is, therefore, of first importance that everyone who encounters a case of accidental intoxication should report his observations with as great accuracy and detail as he can. It is no reflection on the physician or dentist that he should occasionally meet with accidental poisoning from these drugs, but rather is it a reflection that he should be unwilling to publish his own experiences in their fullest detail and aid in the future avoidance of similar accidents.

EXPERIMENTAL

The present investigation was undertaken in the attempt to throw some light upon the causes of acute intoxication in man produced by the several local anesthetics; to discover, if possible, some of the factors which influence the toxicity of these drugs; and to find means of avoiding, or combating, their acute toxic

actions. We have therefore made observations on the following points:

1. The phenomena of acute intoxication after intravenous injection.
2. The maximum toxicity by rapid intravenous injection.
3. The mutual synergism of the several local anesthetics.
4. The toxicity by slow or repeated intravenous injections.
5. The toxicity by subcutaneous injection.
6. The influence of epinephrin on toxicity by subcutaneous injection.
7. Notes on absorption from mucous membranes.
8. Elmination by the liver.
9. Efforts to influence the toxicity of the local anesthetics.
10. Synergism with epinephrin on systematic administration.
11. Measures for resuscitation.
12. Antagonism by ouabain.

More than three hundred experiments were performed; hence we will present only a few typical protocols and give the general results in the form of tables and discussions. We have also drawn upon the results of previous work in our laboratory. All of the experiments were made upon cats, which more closely resemble man in their responses to most drugs than do the rodents, especially with reference to absorption from the subcutaneous tissues. Dogs were not used because, owing to their larger size, we would not have had sufficient supplies of most of the drugs to carry out the investigations planned, and further supplies were unobtainable; and also because we had previously shown that these animals behave essentially as do cats to the local anesthetics. We omitted the use of rabbits, in part, because of their almost prohibitive expense, and because it has been shown that they do not differ essentially from cats in their susceptibility to these drugs by rapid intravenous injection (Schroeder; Hatcher and Eggleston; Smith and Hatcher).

The following local anesthetics were employed, the hydrochlorids being used throughout:[3] alypin, apothesin, beta-eucain,

[3] We are indebted to Prof. Torald Sollmann for having generously provided us with supplies of several of the synthetic cocain substitutes at a time when these were not commercially available.

cocain, holocain, nirvanin, procain (novocain), stovain, tropacocain.

Except where otherwise indicated, all operative procedures were carried out without the use of a general anesthetic. Where it was necessary to expose a vein or an artery the hair was clipped closely and a 10 per cent solution of phenol in oil was used as a local anesthetic. In all experiments, except where stated to the contrary, the local anesthetic was dissolved in 0.85 per cent sodium chlorid solution. In every instance the solutions were prepared on the day on which they were to be used, except in the case of tropacocain, which was sometimes kept in solution for two or three days.

1. Phenomena of acute intoxication after intravenous injection

When a severely toxic, but not fatal, dose is rapidly injected into the femoral vein the general symptoms produced are essentially the same for all of the local anesthetics studied. Within a few seconds there are more or less pronounced stimulation of the respiration and an abrupt fall of blood pressure, the heart rate varying, but usually being slowed. The respiration may then stop for several seconds, or may continue to show stimulation, both rate and depth being increased. Within about a minute the heart begins to improve and the blood pressure rises; at about the same time the respiratory stimulation begins to pass off, and commonly at about this time there is one, or several, brief but severe clonic convulsions. Within less than five minutes the animal is apparently quite normal. The fall in blood pressure is due to a weakening of the heart and not to any immediate vasodilatation. The length of time required for the onset of symptoms does not differ materially for the different drugs or for doses of different size, so long as the dose is a toxic one. There is, however, some difference noted for each of these factors in the rate of recovery. After doses just sufficient to produce the phenomena described complete recovery seldom takes over five minutes, but when the doses are nearly fatal the time of recovery may be about doubled, repeated clonic con-

vulsions being continued for from six to ten minutes after the injection. The different drugs, also, may be divided into two groups with reference to the rate of recovery following a single sublethal dose. Cocain and holocain are the only members of the first group, and are characterized by a decidedly more prolonged action than is seen after any of the other cocain substitutes. This difference will be more clearly shown when we

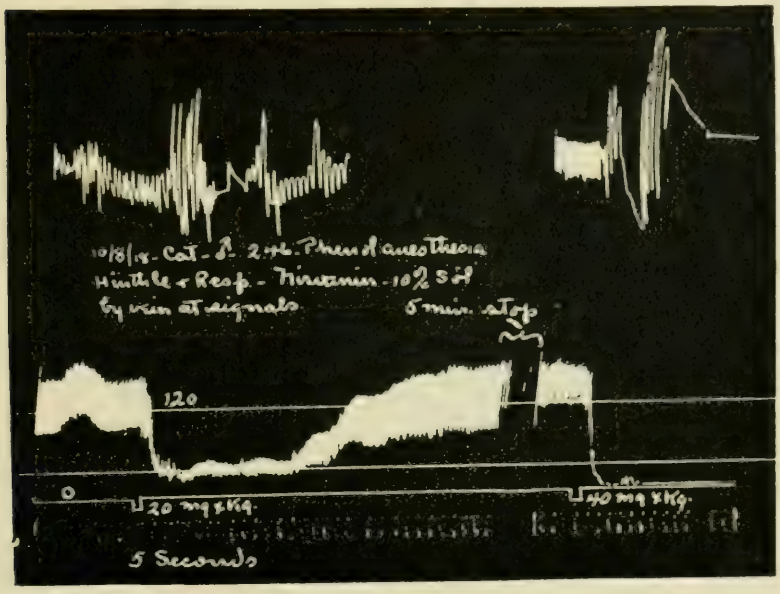


Fig. 1

come to the discussion of repeated doses, and it is one of prime importance. The second group comprises all the other drugs studied; which show only slight differences in the rates of recovery, and from all of which recovery is rapid.

The effects upon the heart and circulation, upon the respiration, and the rapid recovery from these effects, are shown in the tracing—figure 1 which accompanies the following protocol.

Protocol 1. Cat, male, 2.46 kgm.

Twenty milligrams x kgm. nirvanin in 10 per cent solution at once into femoral vein. Sharp fall of blood pressure in less than ten seconds, followed at once by respiratory stimulation. Then respiration almost stopped, while heart began to show slight recovery. Within two minutes after the injection the blood pressure had returned to normal. Clonic convulsions interfered with the respiratory tracing.

Recovery was complete in less than ten minutes; when a surely fatal dose was given and followed by prompt cessation of the heart, transient respiratory stimulation and death.

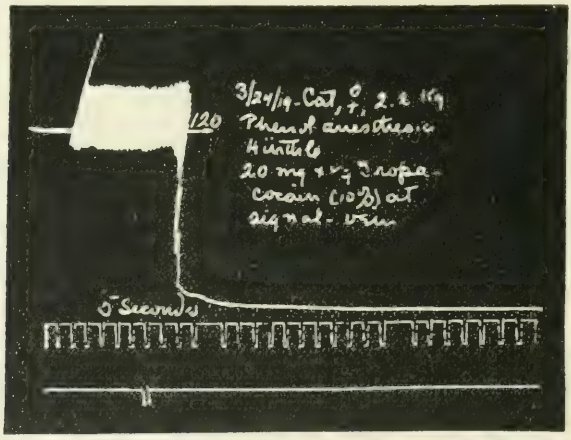


FIG. 2

When fatal doses are given there is often an almost instantaneous general extensor spasm, combined with fixation of the respiration and sudden cessation of the heart beat. After an interval of less than thirty seconds the respiration usually returns in the form of a few shallow gasps, while the heart may resume beating feebly and slowly and continue for a few seconds to several minutes. At other times the auricles alone continue to beat for several minutes, the ventricles not participating, as shown by arterial tracings or by direct inspection of the heart. The abruptness with which the heart may be brought to a standstill is shown in the tracing—figure 2—accompanying the following protocol.

Protocol 2. Cat, female, 2.2 kgm.

Twenty milligrams x kgm. tropacocain in 10 per cent solution at once into femoral vein. Almost instantaneous, violent extensor spasm, respiration fixed; heart stopped suddenly in ten seconds. Few respiratory gasps. Death.

The outstanding features of the phenomena of the toxic actions of all of the local anesthetics, when injected rapidly into the circulation are:

1. The rapidity of onset of the effects upon the heart and respiration.

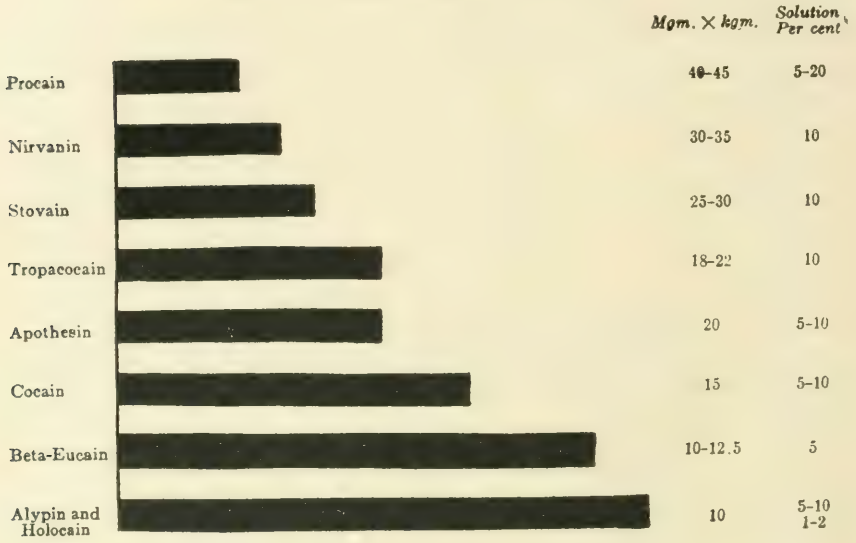
2. The rapidity of complete recovery, even after doses which are sufficient to stop both the heart and respiration, which is true of all of the drugs studied, except cocain, with which the recovery is decidedly slower.

2. Maximum toxicity by rapid intravenous injection

The toxic actions of the local anesthetics being exerted only after the entrance of the drugs into the circulation, we sought to determine the approximate maximum toxicity of each after very rapid intravenous injection of concentrated solutions. We found that it made little difference whether the solution employed was 5 per cent or 20 per cent, since within those limits the dose could be injected from a syringe in from five to fifteen seconds. For the sake of accuracy, however, we append the concentrations employed for each of the drugs. Chart 1 presents in graphic form the relative toxicities of the nine compounds studied; gives the minimal fatal dose in milligrams per kilogram; and shows the range of strengths of the solutions employed. We have taken our figures for procain and stovain in part from our previous papers.

In all cases death is apparently due to the essentially simultaneous paralysis of the heart and respiratory center. In some cases the heart may return and beat feebly for a few seconds after the respiration has failed (see fig. 3), while in others there may be some furtive efforts at respiration after the heart has

CHART 1. SHOWING RELATIVE TOXICITY: FATAL DOSES IN MILLIGRAMS PER KILOGRAM; AND RANGE OF CONCENTRATIONS OF SOLUTIONS FOR THE SEVERAL LOCAL ANESTHETICS



stopped. The direct action on the heart is shown in a tracing taken from a perfused heart by Smith and Hatcher, and in the immediate stoppage of the heart recorded in some of the tracings we reproduce here.

From the chart it is seen that cocain occupies an intermediate position with reference to its maximal toxicity, being surpassed by alypin, beta-eucain and holocain, while the least toxic are nirvanin and procain. It is interesting to note that alypin, which is chemically closely related to stovain, is about three times as toxic, while cocain and tropacocain, which are very closely related, differ appreciably in toxicity, cocain being a third more toxic than tropacocain.

3. Mutual synergism of the several local anesthetics.

The striking similarity noted between the systemic actions of all of these local anesthetics, especially upon the heart and respiratory center, led us to conduct a series of experiments to determine whether or not they are synergistic, and if so to

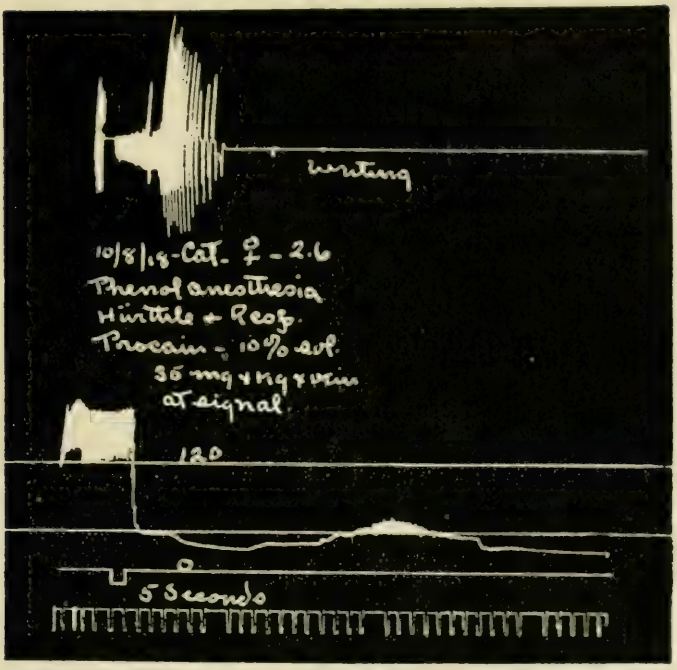


FIG. 3

what extent. The accompanying protocols illustrate our methods of procedure and the results obtained.

Protocol 3. Cat, male, 3.46 kgm.

Twelve milligrams x kgm. nirvanin (37 per cent of fatal dose) plus 18 mgm. x kgm. procain (43 per cent of fatal dose) injected at once into femoral vein in 10 per cent solutions.

Immediate violent respiratory stimulation and distress; heart did not stop; typical rapid recovery.

The total represented 80 per cent of the combined fatal doses.

Protocol 4. Cat, female, 2.2 kgm.

Five milligrams x kgm. alypin (50 per cent of fatal dose) plus 7.5 mgm. x kgm. cocain (50 per cent of fatal dose) at once into femoral vein, in 10 per cent solutions.

Immediate respiratory stimulation; heart stopped in about ten seconds; respiration stopped in about one and a half minutes.

The total represented 100 per cent of the combined fatal doses.

These two protocols are typical of our results throughout, which showed that all of the local anesthetics studied are mutually and quantitatively synergistic. Thus the administration intravenously of a mixture of any two is followed by the general train of symptoms already described, or the administration of a measured fraction of the fatal dose of one plus such a fraction of any other as will make the two fractions equal to 100 per cent of the combined fatal doses of the two results in death with symptoms which are typical of the whole group. Nothing would be gained by the further multiplication of protocols.

4. Toxicity by slow or repeated intravenous injections

We have emphasized, in the section dealing with the maximum toxicity of the local anesthetics by intravenous injection, the fact that the fatal dose is thrown into the blood stream very rapidly—in fact virtually all at once. We have also mentioned the fact that recovery from a sublethal dose is very rapid, except in the case of cocain, even when the dose approaches closely to the fatal. We have also shown in our earlier paper that many times the minimal fatal dose of procain (then called novocain) can be injected intravenously if the injection be made slowly, or if a series of sublethal doses be given at intervals of about fifteen minutes. The same was also shown to be true of stovain by Smith and Hatcher.

In our paper on procain we gave a protocol showing that cocain could not be given by repeated intravenous injection of small doses without the development of evidences of cumulation, as shown by the increasing severity of the symptoms following the later repetitions, and by the ultimate death of the animal after a total of five times the minimum single fatal dose, given in a period of four hours and sixteen minutes in a series of fifteen injections. We have confirmed this observation and have investigated the remaining drugs in this series in the same way,

as well as by slow and nearly continuous injection of relatively dilute solutions. Before discussing the results of this portion of our work we will present two abbreviated protocols.

Protocol 5. Cat, female, 2.46 kgm.

10.17 a.m. 20 mgm. x kgm. nirvanin at once into femoral vein, in 5 per cent solution. Almost immediate convulsion; heart impalpable; enormous respiratory stimulation; followed by prompt recovery.
10.32 a.m. 20 mgm. x kgm. nirvanin as above; symptoms as before, but heart did not stop.
10.47 a.m. 25 mgm. x kgm. nirvanin as above; symptoms as before.
11.07 a.m. 30 mgm. x kgm. nirvanin as above; symptoms as before.
11.30 a.m. 40 mgm. x kgm. nirvanin as above; symptoms as before; but heart stopped almost instantly. After half a minute heart began to beat again.
11.32 a.m. Animal nearly normal.
Recovery complete.

This animal received 135 mgm. x kgm. of nirvanin—more than three times the single fatal dose—in 1 hour and 13 minutes, the last dose being equal to the minimal fatal dose. Recovery was as prompt after the last dose as after any of the preceding. This indicates that the drug was fixed or destroyed at the rate of one fatal dose every twenty minutes.

Protocol 6. Cat, female, 2.55 kgm.

2.32 p.m. Start injection of beta-eucain, 1–1000, into femoral vein.
3.05 p.m. 38 cc. total injected; no symptoms.
3.25 p.m. 60 cc. total injected; slight convulsive movements.
3.40 p.m. 83.7 cc. total injected; no increase in symptoms.
3.45 p.m. Animal released: stands, but is somewhat unsteady from long restraint, otherwise seems normal.

In this case a total of 37.5 mgm. x kgm. of beta-eucain, or nearly four times the fatal vein dose, was injected in one hour and eight minutes without producing serious symptoms. The indicated rate of destruction or fixation is at least as rapid as one minimal fatal dose every twenty minutes.

The rates of destruction or elimination for the several drugs investigated seem to be about as follows: Alypin, beta-eucain,

apothesin, nirvanin, procain, and stovain are destroyed at the rate of at least one fatal vein dose every twenty minutes. Holocain is apparently destroyed a little less rapidly, one fatal dose requiring about half an hour; while cocain is not destroyed more rapidly than about one fatal dose per hour, if as rapidly as that, and the rate of its destruction apparently decreases progressively.

In connection with the elimination of certain of the digitalis bodies we have (15) spoken of the elimination of a poison from those organs upon which its essential, or toxic, actions are exerted as the "essential" elimination to distinguish it from that elimination which may take place immediately after the drug enters the blood stream. In the case of these local anesthetics this "essential" elimination proceeds at a very rapid rate and is accomplished, as we shall show later, by destruction or fixation of the drug. Even in the case of cocain "essential" elimination is rapid at first, although relatively much less so than the remaining local anesthetics. Unlike the digitalis bodies the "essential" elimination of the local anesthetics is virtually the same as the immediate elimination and is practically identical with total elimination, excepting in the cases of cocain and holocain.

This affords an excellent illustration of the difference between those drugs which show the so called "cumulation" and those which do not. In the case of the former the immediate elimination from the blood may proceed very rapidly, but "essential" elimination is a slow process, so that the administration of repeated doses leads to an increasing amount of the drug which is present and acting upon the essential organs. In the case of the latter the administration of repeated doses finds the organs upon which the essential actions are exerted fully restored to normal and quite free from remaining fractions of the previous dose or doses.

5. Toxicity by subcutaneous injection

The relative toxicity of several of the local anesthetics by subcutaneous injection has been studied by LeBrocq (16),

Chadbourne (17), Grode (18), by Schroeder, and by Impens. We have prepared table 1 from the figures given by these authors for the toxicity in rabbits.

Examination of the various doses stated as fatal for the rabbit after subcutaneous injection of cocain, tropacocain and alypin shows a wide range of variation, the largest dose stated for cocain being more than twice the smallest; the largest for tropacocain more than one and three-fourths times as great as the smallest; and the largest for alypin being nearly three times the smallest. Three possible explanations may be offered for such divergence.

TABLE 1

Showing the fatal subcutaneous doses for rabbits of alypin, cocain and tropacocain, according to various authors

ANESTHETIC	FATAL DOSE	AUTHOR
	mgm. x kgm.	
Alypin	115–147	LeBrocq
	110	Schroeder
	50	Impens
Cocain	200	Chadbourne
	153–192	LeBrocq
	100–150	Impens
	87–100	Grode
Tropacocain	over 500	Chadbourne
	281–345	LeBrocq

1. Variation in the susceptibility of the animals.
2. Variation in the concentrations of the solutions used.
3. Variation in the rates of absorption from the subcutaneous tissues.

We are inclined to believe that in view of the rapidity of the destruction of these drugs the latter is the probable explanation. In fact, our observations on the rate of destruction after intravenous injection led us to abandon the effort to fix accurately the fatal subcutaneous doses for the several local anesthetics. We therefore confined these observations to a few experiments with each drug to observe the symptoms produced, the approx-

imate rates of absorption and destruction, and to determine whether or not the relation of destruction to absorption was such as to enable the cat to survive at least five times the average fatal vein dose when injected subcutaneously. A few protocols will illustrate the results obtained.

Protocol 7. Cat, female, 3.34 kgm.

9.40 a.m.	60 mgm. x kgm. cocain in 10 per cent solution subcutaneously.
9.48 a.m.	Emesis.
9.57 a.m.	Clonic convulsions; heart slow and strong; respiration only moderately stimulated . . . . . convulsions repeated nearly continuously.
10.51 a.m.	Convulsions over.
10.57 a.m.	Heart very slow; respiration stops for periods and then returns; is very slow and feeble.
11.07 a.m.	Respiration stopped.
11.10 a.m.	Heart stopped.

Protocol 8. Cat, female, 3.16 kgm.

9.19 a.m.	100 mgm. x kgm. apothesin in 10 per cent solution subcutaneously.
9.58 a.m.	Moderate motor excitation.
9.59 a.m.	Violent general clonic convulsion lasting more than one minute; not repeated.
10.02 a.m.	Animal able to walk but still shows some motor excitation like that seen from morphine.
12.00 m.	Seems quite normal.

Protocol 9. Cat, female, 2.28 kgm.

One hundred and twenty-five milligrams x kgm. tropacocain in 10 per cent solution subcutaneously. No symptoms developed.

Each of the drugs was tried in this way and five, or more than five, times the minimal fatal vein dose was survived when injected subcutaneously in the case of alypin, apothesin, betaeucain, nirvanin, procain, stovain, and tropacocain. Three and

one-third times the fatal vein dose of cocain was survived after subcutaneous injection, while four times the fatal vein dose led promptly to death. Holocain showed a similar high toxicity by subcutaneous injection. It will be recalled that in an earlier section we stated that both holocain and cocain were less rapidly destroyed after intravenous injection than were the other members of the group. Both of these, however, are absorbed rapidly from the subcutaneous tissues so that the rate of absorption may exceed that of destruction with the result that the animal is fatally poisoned by a relatively small dose injected subcutaneously. Absorption of all the other members of the group, though rapid, does not keep pace with the capacity for destruction so that five or more times the fatal vein dose can be administered subcutaneously at one injection without causing death.

With the exceptions of one dose of 150 mgm. x kgm. of stovain and one of 50 mgm. x kgm. of cocain, the time between the injection of the drug and the appearance of the first symptom—where any effects at all were produced—averaged just over twelve minutes, with a minimum of seven and a maximum of sixteen minutes. After the dose of stovain cited as an exception the interval was twenty-five minutes, while after that of cocain it was twenty-eight minutes. A dose of 60 mgm. x kgm. of cocain, however, was followed in eight minutes by the first symptoms of its systemic action. When the doses given produced convulsions as part of their toxic actions the average interval between the administration of the dose and their onset was a little more than twenty-two minutes, with a minimum of nine and a maximum of eighty minutes. The eighty minute interval occurred following the dose of stovain just cited as requiring twenty-five minutes before the appearance of the first symptom and is the only one greater than twenty-five minutes. If it be omitted from the calculation of the average time to convulsions, the latter falls to fourteen minutes. One instance has not been included in either of the preceding computations, namely, that of a dose of 50 mgm. x kgm. of cocain. In this case no convulsions were observed in five hours, but more than twenty-seven hours later the animal had one mild clonic convul-

sion. This experiment is so strikingly at variance with all of the others that we append the protocol.

Protocol 10. Cat, female, 2.6 kgm.

February 14,	10.26 a.m.	50 mgm. x kgm. cocain in 5 per cent solution subcutaneously.
	10.54 a.m.	Emesis, repeated.
	2.44 p.m.	Emesis.
	5.00 p.m.	Animal has been depressed all afternoon.
February 15,	9.00 a.m.	Still somewhat depressed, refuses food.
	2.00 p.m.	Mild clonic convulsion; not repeated.
February 16,	9.00 a.m.	Apparently normal.

In this experiment absorption began fairly promptly for emesis appeared twenty-eight minutes after the injection, and continued more slowly, for emesis was repeated at intervals for the next four hours. It is quite evident, however, that, slow as was the absorption, "essential" elimination was not able to keep pace with it for a clonic convulsion appeared twenty-seven hours after the injection, and we have not seen convulsions develop after intravenous injection of less than about two-thirds of the fatal dose. In this instance "essential" elimination was much slower than one fatal vein dose per hour, as stated to be the usual approximate rate after intravenous injection, and illustrates the difference between cocain and the other members of the group.

Reference to the review of the literature of intoxication in man shows that the symptoms are much the same as those here recorded for cats; that they do not differ materially in nature for the several drugs; that the interval between the injection of the drug and the onset of symptoms is generally very brief in man, as in animals; and, finally, that recovery in man is quite rapid with all of the drugs except that after cocain it may be decidedly prolonged, just as in the case of cats. Thus after all of the drugs used, except cocain, recovery in man was almost invariably complete in an average of a little over two hours, the limits being half an hour and three hours and forty minutes. No attempt has been made to fix the limits of the several time

intervals for cats with great accuracy, gross differences between the several members of the group being alone of importance. Therefore the figures stated are not to be taken as exact indices of either the rapidity of absorption or of duration of action. Such a determination would have required far larger amounts of most of the drugs than we had at our disposal, and would have added little, if anything, of importance to the results.

We did, however, pursue this phase of the investigation further by observing the effects of giving repeated subcutaneous doses of several of the drugs at intervals of fifteen minutes. The results are shown in the accompanying protocols.

Protocol 11. Cat, male, 2.16 kgm.

10.04 a.m.	5 mgm. x kgm. holocain in 2 per cent solution subcutaneously; dose repeated at 10.19 and 10.34 a.m.
10.39 a.m.	Emesis, depression, crying, tremulous.
10.48 a.m.	Mild general convulsion, not repeated.
11.30 a.m.	Seems fairly normal, sits up.
3.00 p.m.	Eats ravenously, is entirely normal.

Protocol 12. Cat, female, 2.60 kgm.

10.06 a.m.	10 mgm. x kgm. cocain in 10 per cent solution subcutaneously; dose repeated at 10.21 and 10.36 a.m.
10.41 a.m.	Nausea, slightly tremulous, pupils dilated.
10.51 a.m.	10 mgm. x kgm. as before.
11.02 a.m.	Very hyperexcitable, almost in convulsions.
11.21 a.m.	10 mgm. x kgm. as before, no convulsions having developed.
11.30 a.m.	Mild general clonic spasm, followed by tetanic spasm.
11.56 a.m.	Spasms repeated up to now; animal now sitting up, head shakes continuously.
2.50 p.m.	Emesis; is depressed.
4.00 p.m.	Still depressed, refuses food, no other symptoms.
	Normal following morning.

Protocol 13. Cat, female, 3.06 kgm.

10.00 a.m.	20 mgm. x kgm. apothesin in 10 per cent solution sub-

cutaneously. Dose repeated at 10.15, 10.30, 10.45, 11.00 11.30, and 11.45 a.m. and at 12.00 noon.' Slightly tremulous and showed slight motor excitation after the fifth and seventh doses.

12.15 p.m. 30 mgm. x kgm. as before.

12.21 p.m. Slightly tremulous, otherwise normal. No further symptoms.

These protocols illustrate the differences already mentioned between the relative rates of absorption and destruction of holocain and cocain as compared with other of the local anesthetics, including nirvanin, procain and stovain, protocols of which have been omitted. The subcutaneous administration of only one and one-half times the fatal vein dose of holocain in three equal portions during thirty minutes was followed by a convulsion, and recovery seemed about complete an hour after the last dose. Three and one-third times the fatal vein dose of cocain, in five equal doses in one hour and fifteen minutes, produced serious intoxication and recovery was not complete after a lapse of four hours following the last dose.

A further, and striking, illustration of the slow "essential" elimination of cocain is to be found in Grode's paper. He tried to habituate various animals to cocain by repeated subcutaneous injection and he noted, in both cats and dogs, what he called an increased susceptibility to the later repetitions of a fixed dose, when these were made at intervals of one or two days. Thus, in one cat to which he attempted to give a daily subcutaneous dose of 20 mgm. x kgm. of cocain, the first dose caused salivation only; the second caused emesis; the third, three tetanic spasms; the fifth, eight tetanic spasms; then a two day interval intervened between the sixth and seventh doses, but the seventh dose caused repeated spasms and the eighth, on the following day, proved fatal in fifteen minutes.

In this experiment of Grode's it is to be noted that the cat was unable to destroy or eliminate as much as one and one-third times the fatal vein dose in the course of twenty-four hours when injected subcutaneously. This is a much slower rate of "essential" elimination than was indicated by our experiments

in which cocain was injected intravenously, and the difference is not readily explained.

In contrast with these we have administered about seven times the fatal vein dose of nirvanin in one and three-quarter hours, nine times the fatal vein dose of procain in two hours, and nine and one half times the fatal vein dose of apothesin in two and a quarter hours, without the production of convulsions in any instance. In all of these experiments, even after such very large doses, recovery was essentially complete within three hours and a half.

6. Influence of epinephrin on toxicity by subcutaneous injection

The results of the experiments discussed in the preceding section made us feel quite certain that the simultaneous injection of epinephrin and any of the local anesthetics would delay the onset of symptoms and diminish the toxicity of a given dose of the anesthetic by virtue of delayed absorption.

The results of all these experiments may be briefly summarized by saying that no symptoms followed doses of 100 and 150 mgm. x kgm. of nirvanin, 100 mgm. x kgm. of apothesin, or 50 mgm. x kgm. of alypin when injected subcutaneously in solutions containing 0.05 mgm. x kgm. of epinephrin in 1–10,000 dilution. A dose of 150 mgm. x kgm. of stovain with epinephrin caused nausea and emesis only, and they did not appear until one hour after the injection. A dose of 50 mgm. x kgm. of holocain with epinephrin was followed in thirteen minutes by the onset of violent clonic convulsions which were repeated many times during about five hours. The animal, however, recovered completely during the night and was apparently normal on the following morning. We have previously shown that the simultaneous administration of epinephrin greatly reduces the toxicity of procain by subcutaneous injection. In one experiment with alypin, the dose duplicating that previously mentioned, emesis followed thirty-seven minutes after the injection and clonic convulsions appeared two minutes later and were repeated for nearly an hour and a half. It is not clear why the epinephrin

failed to have a greater effect in delaying the absorption in this case, unless it be that this animal was more susceptible than usual to the irritant actions of the alypin. Finally, a dose of 85 mgm. x kgm. of cocain, with epinephrin, produced no symptoms in more than three hours, but the following day—eighteen and a half hours later—the animal was found in clonic convulsions which continued for four hours, up to the death of the cat.

These experiments also show that cocain and holocain are relatively slowly destroyed, so that even when absorption is delayed by the vasoconstrictor action of epinephrin they are still capable of accumulating in the essential organs to cause serious intoxication or death when five to six times the fatal vein dose is injected subcutaneously. On the other hand, the importance of the rapid destruction of the other members of the group is emphasized by these experiments with epinephrin, and it would seem to point to the decided value of always using epinephrin, as a routine, in combination with the cocain substitutes for subcutaneous injection in man to delay absorption and diminish the chances of accidental poisoning.

7. Notes on absorption from mucous membranes

Some experiments were performed to determine whether or not the local anesthetics are rapidly absorbed from the mucous membranes, especially those of the nose, urethra and bladder. Cocain and procain were employed chiefly, though a few experiments were made with apothesin. At first we attempted to apply a measured amount of a solution of procain to the cat's nasal mucous membrane by the insertion of a series of cotton plugs saturated with the solution. The small size of the nasal spaces, however, showed this method to be unsatisfactory and we therefore resorted to plugging the nares as far back as possible with small pledgets of cotton saturated with the solution to be tested. Then, with the cat restrained in a box and held with the head upward, small drops of the solution were instilled at frequent intervals into each nasal cavity until the desired amount had been introduced, or until symptoms developed.

The one objection to this method is that it does not entirely prevent some of the solution from running back into the pharynx and being swallowed. The following is a typical protocol.

Protocol 14. Cat, female, 2.8 kgm.

9.32–9.39 a.m.	53 mgm. x kgm. cocain in 10 per cent solution instilled into nose.
9.49 a.m.	Tremulous, pupils dilated.
9.51 a.m.	Clonic convulsion; repeated several times.
9.58 a.m.	No further convulsions.
10.20 a.m.	No evident symptoms.
11.05 a.m.	Emesis.
1.00 p.m.	Apparently normal.

Repeated clonic convulsions followed immediately after the instillation of 212 mgm. x kgm. of procain in 20 per cent solution into the nose of another cat, but the animal had recovered completely in two and a half hours. A third cat showed no symptoms after the instillation of 133 mgm. x kgm. of procain, partly into the nares and partly into the conjunctival sacs. In a fourth experiment 112 mgm. x kgm. of apothesin in 10 per cent solution were instilled into the nose, and the cat vomited once about half an hour later, but showed no other symptoms. While absorption was sufficient to permit of the development of some evidences of intoxication in three of the four experiments, it must be borne in mind that the doses employed were massive; thus the dose of cocain corresponds to 53 grains, or 3.5 grams, for a man of average size; that of procain which produced convulsions, to 212 grains, or nearly 14 grams; and that of apothesin, to 112 grains, or about 7 grams, for man. It is not surprising that some symptoms should have resulted from such doses, but rather that the intoxications produced were so mild as recorded.

Several instances of acute poisoning in man have been reported following the instillation of each of a number of the local anesthetics into the male urethra, and so frequent have been such cases following the use of cocain that special warning is given by many writers as to the dangers of thus anesthetizing

the urethra. This would seem to indicate rapid absorption from the urethral mucosa, but it is noteworthy that among the six cases of poisoning in man after intraurethral instillation of beta-eucain or alypin, which we have cited from the literature, the urethral mucous membrane had been damaged in four. Such a condition would naturally be expected to hasten the absorption, especially in such a highly vascular structure, and when the solution is introduced under considerable pressure, as is the case in most of such instillations. We undertook to study the absorption of some of these drugs from the urethra of the male cat, but found its capacity too small, as shown by the protocol of a control experiment.

Protocol 15. Cat, male, 2.16 kgm. (control)

Lightly anesthetized with ether, bladder exposed, blunt needle tied into anterior urethra and a concentrated solution of methylene blue instilled slowly. When 0.1 cc. of the solution had been injected it was seen to appear in the bladder, showing the small capacity of the cat's urethra.

It was evident from this experiment that large doses of the local anesthetics could not be kept within the cat's urethra, even when very concentrated solutions were employed, and that the injection was chiefly into the bladder. Nevertheless, the solution was made to pass through the urethra, and some of it did remain there, so we injected several cats in this way with large doses of the anesthetics. The results were invariably negative, that is, no absorption was evident in any of the seven experiments in which amounts of procain up to 150 mgm. x kgm.; of cocain, up to 90 mgm. x kgm.; and of apothesin, up to 100 mgm. x kgm. were thus instilled through the urethra into the previously emptied bladder. The entirely negative results of these experiments led us to abandon their further repetition.

We performed one experiment to discover whether the cat would absorb appreciable amounts of procain from the vagina. A blunt needle was inserted into the vagina of an unanesthetized cat and the orifice was plugged with cotton saturated with petro-

latum. Five hundred milligrams, or 277 mgm. x kgm., of procain in 50 per cent solution were instilled. No symptoms resulted.

These experiments on absorption from the mucous membranes indicate: That the local anesthetics are not more rapidly absorbed from the naso-pharyngeal mucous membranes of the cat than from the subcutaneous tissues. That in the cat the bladder, urethra and vagina absorb the local anesthetics very poorly.

8. Elimination by the liver

Wiechowski (19) sought to determine the fate of cocain in the animal organism by administering large doses orally to dogs and rabbits and examining the urine for cocain and ecgonin after collection for forty-eight hours. He found that dogs excreted an average of 5.1 per cent of the dose administered, the amounts recovered in the urine varying between none and 12.3 per cent. Rabbits, on the other hand, excreted none at all in the urine. He added cocain to the hashed livers or muscles, freshly excised, and agitated the mixtures for periods of four hours at the end of which he recovered about 80 per cent of the cocain originally added. From these experiments he concluded that the destruction of cocain should be regarded as a vital phenomenon. He also extracted the liver of a dog, weighing 5.8 kgm., which died half an hour after the administration of a total of 0.3744 gram of pure cocain. One hundred and eighty-three grams of the liver yielded 8 mgm. of cocain, an amount which corresponded to that which would have been present after uniform distribution of the drug throughout the body. He further determined that ecgonin does not appear in the urine of the dog or rabbit in appreciable amounts as a decomposition product of cocain.

Rifâtwachdani (20) obtained quite different results for the excretion of cocain in the urine of rabbits after subcutaneous injection. He found that rabbits excreted in the urine in twenty-four hours amounts varying from 42 to 85 per cent of a single subcutaneous dose of 50 mgm. of cocain hydrochlorid, showing absence of marked destruction. The excretion of cocain was

studied in one rabbit receiving a daily subcutaneous dose of 50 mgm. of cocain. The amounts recovered in the urine on successive days rose from 33.6 to 112.1 per cent of the daily dose. In the whole experiment 65.8 per cent of the cocain administered was thus excreted. No ecgonin was found in the urine. The author also cites and confirms Kohlhardt's observation that long contact of cocain with the tissues, as by injection into a leg the circulation of which had been interfered with by constriction with a tourniquet, leads to a great reduction in its toxicity when the constriction is released. He tried to extract the cocain from such previously constricted legs, amputated two or three hours after the injection, and recovered from 20 to 100 per cent of that injected. He holds that this shows conclusively that there is no destruction or detoxification of cocain by the tissues, and suggests that the reduction in toxicity following injection into the constricted leg may be due to slow absorption and entrance into the general circulation, as is known to be the case with strychnin.

We showed in a previous paper that procain is rapidly destroyed during its perfusion through the cat's liver, and the same was shown to be true of stovain by Smith and Hatcher. In these two papers it was also shown that neither procain nor stovain is excreted in the urine of the cat in appreciable amounts. The results of the perfusion experiments were so clear cut and definite in demonstrating the destruction or fixation of these two local anesthetics by the liver that we decided to study the fate of several other members of the group in the same way. Two abbreviated protocols illustrate the methods of procedure and the results obtained.

Protocol 16. Cat, female, 3.44 kgm.

Under light ether anesthesia the animal was bled from the carotid artery while normal saline solution was being injected into a femoral vein. After defibrination the diluted blood measured 200 cc. In this we dissolved 600 mgm. of cocain hydrochlorid and divided the whole into a portion measuring 20 cc., which was reserved for control tests, and one measuring 180 cc.,which was used for perfusing the liver.

The liver of the cat was removed at once after exsanguination, a cannula was tied into the portal vein and all the remaining blood was washed out with fresh, warm normal saline.

The liver was then perfused with the 180 cc. of diluted defibrinated blood, containing 540 mgm. of cocain, for one hour at a temperature of 37°C. The perfusion fluid passed through the liver more than 35 times, being oxygenated frequently in the intervals.

After perfusion 180 cc. of fluid were recovered, some dilution of the original having taken place with the saline which remained in the liver after washing.

Both the perfused fluid and the sample of defibrinated, diluted blood which was reserved for control were tested on cats by intravenous injection.

Tests

Control blood: Cat, male, 2.58 kgm.

11.20 a.m. 6 cc. x kgm. of the control blood were injected rapidly into femoral vein. Immediate respiratory stimulation, violent clonic convulsions, repeated every few minutes until 11.40 a.m.

11.50 a.m. Killed with chloroform.

Perfused fluid: Cat, male, 2.12 kgm.

11.27–11.28 a.m. 7.78 cc. x kgm. perfused fluid injected into femoral vein. Respiratory stimulation, one clonic convulsion.

11.29–11.31 a.m. 6.83 cc. x kgm. additional injected. One clonic convulsion.

11.33–11.34 a.m. 4.69 cc. x kgm. additional injected. Violent clonic convulsions began and were repeated just as in the control test.

Calculation

6.0 cc. of control blood contained 15 mgm. cocain.

19.3 cc. of perfused fluid, equivalent to 6.0 cc. of control, contains 15 mgm. of cocain.

180.0 cc. of perfused fluid contains 138.6 mgm. cocain.

Since 540 mgm. of cocain were originally present in the 180 cc., 401 mgm. were destroyed in the liver during perfusion.

Since the original perfusion fluid was somewhat diluted during passage through the liver, this figure is a little too high, but the result of

the experiment is to show conclusively that the liver destroyed or fixed not less than half of a total of 540 mgm. of cocain during perfusion for one hour, or an amount corresponding to about 5 fatal intravenous dose as for the cat from which the liver was removed.

Protocol 17. Cat, male, 3.56 kgm.

Bled and liver prepared as in preceding, and 1 gram of tropacocain, dissolved in a little normal saline, was added to the defibrinated blood and the whole made up to 250 cc. Portion of 50 cc. reserved for control tests; 200 cc. used to perfuse the liver.

The perfusion was carried on for half an hour, the fluid flowing through the liver as fast as possible. At least 40 complete circuits were made.

A test of the control fluid showed no destruction, while one of the perfused fluid indicated practically total destruction of the entire 800 mgm. of tropacocain originally present, or an amount equivalent to more than 10 times the single fatal vein dose for the animal from which the liver was removed.

The unused portion of the control sample of defibrinated blood was extracted and the extract tested on a cat. The test indicated recovery of practically all of the tropacocain originally present.

The liver, after perfusion, was then ground and extracted by the same method as that employed for the blood and tested on a cat. The test of the extract corresponding to one-third of the entire liver failed to reveal the presence of any tropacocain.

Perfusions similar to these were carried out with apothesin and nirvanin and showed that practically all of a total of 500 mgm. of apothesin and about two-thirds of a total of 900 mgm. of nirvanin were destroyed[4] by the liver during perfusion for one hour. In all, six of the nine different local anesthetics have been tested by perfusion through the excised liver and in every case it has been demonstrated that that organ is capable of destroying large amounts of these drugs rapidly. It is scarcely necessary to

[4] We do not know with absolute certainty that the local anesthetics are actually destroyed in the liver, in the sense of chemical decomposition. There is a possibility that they may merely be fixed in an insoluble, or inert, form. All of the evidence, however, points to actual destruction by decomposition and we therefore prefer to speak of these drugs as being destroyed.

remark that we made control experiments to exclude destruction or other form of detoxification of the drugs by defibrinated, diluted blood, kept at body temperature and oxygenated during periods of time similar to those elapsing in the perfusions.

This rapid destruction of the local anesthetics by the liver accounts not only for the rapid recovery of the intact animal from the intravenous injection of doses which are just sublethal, but also for the fact that with most of the members of the group such doses can be repeated indefinitely at intervals of about twenty minutes until many times the single fatal dose has been injected. This destruction also explains the fact that when just sublethal doses are injected intravenously, even though respiration may have been stopped for one or two minutes, the animal will recover if the heart continues to beat and circulate the blood through the liver. The rapid destruction of these drugs also serves to explain in part the success of some of the resuscitative measures to be described later, as well as the ultimate recovery from more than average lethal doses after the previous administration of a cardiac stimulant drug.

It seems certain that man is also capable of rapidly destroying the anesthetics in his liver and that this largely accounts for his rapid recovery from severely toxic symptoms. Such destruction is a matter of prime importance in connection with the employment of antagonistic and resuscitative measures in cases of acute intoxication in man, for it is evident that if the vital functions of respiration and circulation can be maintained for even a short time there is always the chance of complete recovery through destruction of a portion of the drug which has been absorbed.

9. Efforts to influence the toxicity of the local anesthetics

One of the principal objects of our investigation of the local anesthetics having been the endeavor to throw some light upon the factors which favor acute intoxication in man and the methods of avoiding or overcoming accidental poisoning, we systematically investigated a number of agents and measures

which might be anticipated to exert some influence on the toxicity of these drugs. In many instances one or two experiments were sufficient to convince us that a lead was not worth following, so that we abandoned further work at once. In others we felt that we should make our experiments somewhat more extensive and searching, even though the results at first were negative.

(*a*) *Influence of atropin.* We performed a series of thirteen experiments to determine whether or not the intravenous injection of atropin just before that of one of the anesthetics would influence the toxicity of the latter through paralysis of the vagus endings in the heart or stimulation of the respiratory center. The dose of atropin employed was either 2 or 5 mgm. x kgm. and the interval between its injection and that of the local anesthetic varied from three to ten minutes. Cocain, stovain, procain and apothesin were tried as representatives of the group, doses both larger and smaller than those stated as the average fatal were administered. There was no evidence of any influence of atropin upon the symptoms produced by the local anesthetics, or upon the size of the fatal vein dose.

(*b*) *Influence of sodium bicarbonate.* Gros (21) showed that the addition of sodium bicarbonate to the commonly used salts of the anesthetic bases enhanced their anesthetic activity, due, he believed, to the better penetration of the nerves by the liberated bases than by their salts. Sollmann (22) summarizes a series of his own investigations and generally confirms Gros so far as the increase of anesthetic activity by the addition of sodium bicarbonate is concerned, but states that it is not certain whether such addition modifies their toxicity or not. We therefore investigated the influence of sodium bicarbonate on the toxicity of the various drugs of the group.

In all of the experiments the mixture of the local anesthetic with the sodium bicarbonate was made in the syringe just before injection, or in a graduate, and the solution, which usually showed some cloudiness or a fine precipitate, was vigorously shaken and drawn into the syringe for immediate injection. In every instance the dose of the bicarbonate was 25 mgm. x

kgm. in the form of a 5 per cent solution, while the local anesthestic was used in the customary concentration. All injections were made intravenously at the usual rate. The doses of the local anesthetics were usually 75 per cent of the average fatal, though in some cases the fatal doses were used, and once or twice doses larger than the average fatal were employed to determine whether there was a reduction in toxicity. In a few experiments repeated large doses of the anesthetic were given along with the alkali. The results, including the number of experiments performed, are shown in table 2.

TABLE 2

Showing results of simultaneous intravenous injection of local anesthetics with 25 mgm. x kgm. of sodium bicarbonate in cats

ANESTHETIC	NUMBER OF EXPERIMENTS	DOSES OF ANESTHETIC PER CENT OF FATAL	EFFECT ON TOXICITY
Procain	6	60–120	None
Nirvanin	3	80–100	None
Cocain	3	80–100	None
Apothesin	2	75	No increase
Stovain, beta-eucain, alypin, tropacocain	1 each	75	No increase

(c) *Influence of caffein.* Caffein has been employed clinically in the treatment of acute cocain poisoning and Bastedo (23) cites Lieb as having found caffein effective in removing cocain heart block in the isolated turtle heart. It seemed possible, therefore, that it might prove somewhat antagonistic to the toxic actions of the local anesthetics in cats. Doses of 10 mgm. x kgm. of caffein were slowly injected intravenously and three minutes later one and a quarter times the minimal fatal dose of the local anesthetic to be tested was injected in the usual manner. Cocain, procain and apothesin were tried, but in no instance was there any evidence of antagonism, even when artificial respiration was employed to give the heart a chance to recover, nor was there alteration in the typical response to the local anesthetic. In one experiment we injected 3 mgm. x kgm. of caffein and 50 mgm. x kgm. of procain in the same solution with the

usual prompt onset of symptoms, followed by death in the typical manner.

(d) Influence of hydrated chloral. We noted in our previous paper two instances in which cats died promptly after the intravenous injection of as little as 9 and 10 mgm. x kgm., respectively, of procain, given during deep chloral narcosis. This suggested that hydrated chloral increases the toxicity of the local anesthetics, hence we decided to investigate the matter further. A series of nineteen experiments were performed and the results are shown in table 3.

These experiments indicate that severe poisoning by hydrated chloral may increase the susceptibility of the cat to the toxic actions of the local anesthetics, occasionally to a marked degree. On the other hand, degrees of poisoning by chloral short of the most severe do not as a rule seem to have much influence on the susceptibility to the local anesthetics. That the previous administration of hydrated chloral does exert some influence, both upon the immediate response to the local anesthetics, and upon

TABLE 3

Showing influence of hydrated chloral on toxicity of the local anesthetics for the cat

ANESTHETIC	DOSE		RATE OF INJECTION	NARCOSIS*	RESULT
	Mgm. x kgm.	Per cent of fatal			
Procain	9.0	21	5 minutes	Deep	Death
	10.0	24	Rapid	Deep	Death
	30.0	71	Rapid	Moderate	Recovery
	48.9	119	28 minutes	Deep	Recovery
	106.0	252	128 minutes	Deep	Recovery
Cocain	10.0	66	Rapid	Deep	Recovery
	10.0	66	Rapid	Deep	Recovery
	12.5	83	Rapid	Deep	Death
Tropacocain	15.0	75	Rapid	Mild	Recovery
	15.0	75	Rapid	Mild	Death
	15.0	75	Rapid	Mild	Death
Stovain	25.0	99	Rapid	Moderate	Death
	27.0	100	Rapid	Moderate	Death

TABLE 3 *Continued*

The following cats received repeated doses, all injections rapid

CAT	ANESTHETIC	DOSE		INTERVAL IN MINUTES	NARCOSIS	RESULT
		Mgm. x kgm.	Per cent of fatal			
A	Apothesin....	10.0	50		Mild	Recovery
		10.0	50	20		Recovery
		5.0	25	20		Recovery
B	Apothesin....	10.0	50		Mild	Recovery†
		10.0	50	15		Recovery
		15.0	75	15		Recovery
C	Apothesin....	15.0	75		Mild	Recovery
		15.0	75	20		Recovery
		15.0	75	20		Recovery
D	Stovain	20.0	72		Deep	Recovery
	Procain....	35.0	83	20		Death
E	Stovain.....	20.0	72		Moderate	Recovery
	Apothesin..	15.0	75	22		Recovery
F	Apothesin	15.0	75		Moderate	Recovery
	Procain....	35.0	83	30		Recovery

* Deep narcosis is of such a degree that the animal cannot be roused; moderate is such that the animal can be roused partially by very rough handling and repeated painful stimuli, but lapses into sleep at once when left; mild is such that the animal remains in deep sleep when undisturbed, but can be roused fairly readily by rough handling, returning to deep sleep only after an interval of a few minutes.

In this connection we wish to emphasize the degree of intoxication produced by the chloral, even when classed as mild. Its severity is indicated by the fact that of 30 cats to which chloral was administered to produce narcosis, five died in their cages before the time for use and four died as a result of the handling incident to the operation.

† This animal received chloral the day preceding the injection of the anesthetic and was used without a further dose of chloral because it remained in a state of moderate to mild narcosis.

the rate of recovery is, however, clearly shown by the results of two parallel experiments, illustrated by figures 4 and 5. The figures carry all the data except the times required for complete recovery in the chloralized cat, which were approximately ten minutes after each of the three doses, contrasting with periods

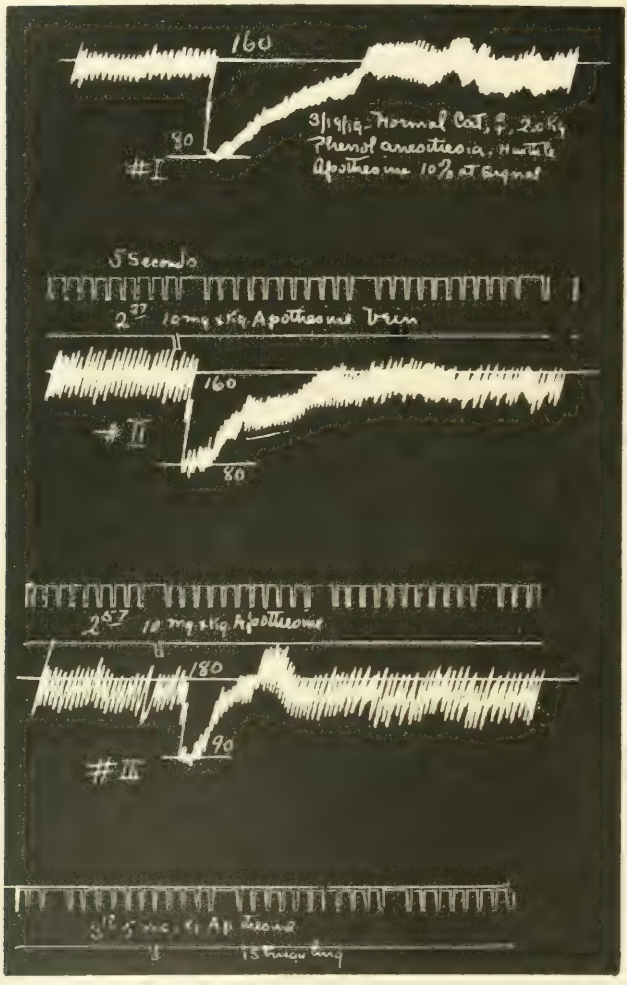


FIG. 4

of less than three minutes in the normal cat. The tracings show that after chloral the fall in blood pressure following the injection of the anesthetic is decidedly less abrupt than in the normal control, and while this effect was not always seen, it still was of frequent occurrence in those experiments in which we took graphic records. The slower recovery is almost certainly due to

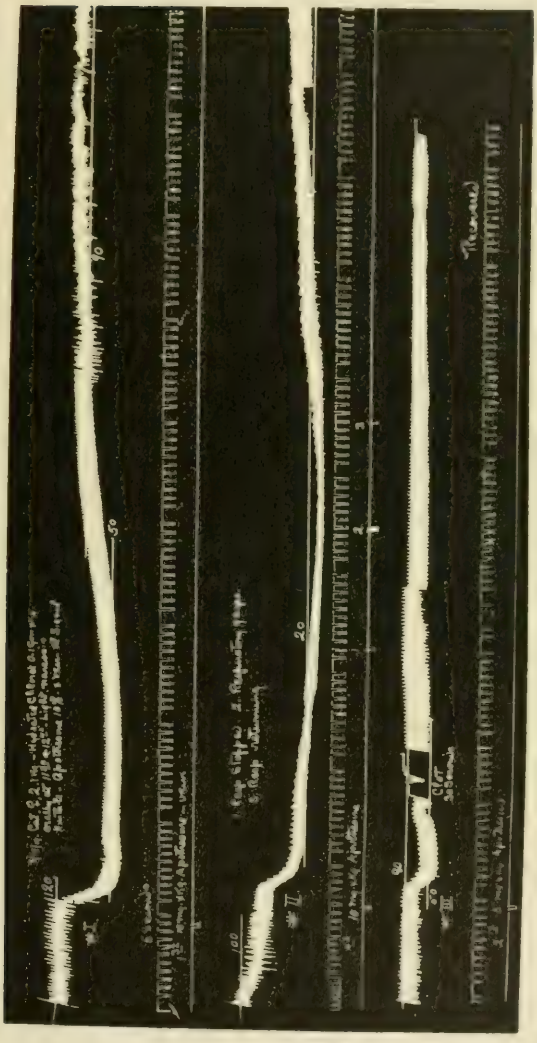


FIG. 5

interference with the circulation whereby a longer time is required for destruction in the liver.

It is surprising that chloral did not show a more decided influence on the ultimate recovery from large doses of the local anesthetics, or upon the size of the fatal doses, in view of the material delay in the initial rate of recovery as shown in the blood pressure curves, and in view of the lower initial blood pressure in the chloralized than in the normal cat.

(*e*) *Influence of hemorrhage.* Attention has been called by several clinical writers to the fact that acute intoxication by the local anesthetics seems to be rather more likely to occur in ill-nourished, anemic persons than in the robust. A perusal of the literature, of accidental acute poisoning, however, does not show that the anemic and ill-nourished supply any more such occurrences than do others. Nevertheless, we investigated the influence of severe acute hemorrhage on the toxicity of several of these drugs by intravenous injection. The experimental method consisted in bleeding the cat from an artery to the extent of one-fourth of the total calculated blood volume. In from two to five minutes after the bleeding the anesthetic was injected into the femoral vein. The general results are shown in table 4.

Of the eleven cats receiving from 75 to 80 per cent of the fatal vein dose of the local anesthetics, seven died after typical symptoms and four recovered normally. Three cats receiving repeated injections, on the other hand, recovered from both doses in a manner indistinguishable from normal cats. It would seem, therefore, that severe acute hemorrhage may be regarded as diminishing the cat's ability to recover from large fractions of the average fatal vein dose of the local anesthetics in about half the instances. It is surprising that the effect of such a measure as the rapid withdrawal of one-fourth of the cat's total blood is not greater, for such a procedure not only materially reduces the blood pressure, but owing to the reduced blood volume tends to impair the function of all of the organs including the heart, the central nervous system and the liver. Such impairment might naturally be expected to both increase the cat's susceptibility to the local anesthetics and to reduce its capacity for destroying them in the liver.

TABLE 4

Showing the results of intravenous injection of local anesthetics in cats previously deprived of one-fourth of their total blood volume

ANESTHETIC	DOSE		RESULT
	Mgm. x kgm.	Per cent of fatal	
Cocain	12.5	83	Typical symptoms; death
	12.0	80	Typical symptoms; death
	12.0	80	Typical symptoms; recovery
	11.0	73	Typical symptoms; death
Apothesin	20.0	100	Typical symptoms; death
	15.0	75	Typical symptoms; recovery
Stovain	22.5	89	Typical symptoms; death
	20.0	72	Typical symptoms; recovery
Tropacocain	15.0	75	Typical symptoms; death
	15.0	75	Typical symptoms; death
	10.0	50	Typical symptoms; recovery

Each of the following cats received two doses

CAT	ANESTHETIC	DOSE		INTERVAL IN MINUTES	RESULT
		Mgm. x kgm.	Per cent of fatal		
A	Apothesin	10.0	50		Typical symptoms; recovery
		15.0	75	20	Typical symptoms; recovery
B	Apothesin	15.0	75		Typical symptoms; recovery
		15.0	75	30	Typical symptoms; recovery
C	Stovain	18.0	64		Typical symptoms; recovery
		22.0	88	35	Typical symptoms; recovery

(*f*) *Miscellaneous.* We planned to investigate the effects of nervous exhaustion in cats upon the toxicity of the anesthetics and sought to produce exhaustion by prolonged, intense excitement. We administered doses of 10, 10 and 20 mgm. x kgm. of procain at intervals of ten and fifteen minutes, respectively, to one such exhausted cat, but saw no definite evidence of an increased susceptibility to the drug. We then resorted to the

administration of camphor in doses sufficient to produce violent clonic convulsions which were repeated frequently during periods of two or more hours. When the convulsions were becoming infrequent and greatly diminished in severity owing to exhaustion we administered various doses of the local anesthetics, including procain, cocain, stovain and tropacocain, but observed neither increased susceptibility from the exhaustion, nor increased tolerance from the stimulant actions of the camphor.

We also sought to test the influence of surgical shock but after a single experiment we abandoned further efforts. In this experiment the cat was anesthetized lightly with ether and all of the measures commonly employed for producing shock were applied during a period of three-quarters of an hour, at the end of which time the cat's blood pressure had fallen only 28 per cent. The intravenous injection of 75 per cent of the fatal dose of apothesin resulted in death after four minutes, the respiration continuing feebly for nearly two minutes after the heart had stopped. No convulsions were observed following the dose of apothesin. While the result in this experiment is suggestive of an increased susceptibility due to the surgical shock, we did not feel justified in attempting its repetition owing to the difficulty in producing shock and to the extremely abnormal condition of the animal as a result of the manipulations.

Finally we sought to determine the influence of hypertonic saline in delaying the penetration of the local anesthetics into the cells and performed two experiments in which 25 cc. x kgm. of a 10 per cent solution of absolute sodium sulphate were injected intravenously just before the injection of procain, but there was no indication that this measure had any influence upon the toxicity of the anesthetic.

10. Synergism with epinephrin on systemic administration

It is well known that cocain and epinephrin have a synergistic action on the blood pressure, and we have called attention to the fact that procain is similarly synergistic with epinephrin, although, unlike cocain, it causes a fall in the blood pressure

when given alone. All of the other substitutes for cocain which we have investigated also cause a fall in blood pressure, and produce local vasodilatation when injected subcutaneously. We have tried all of the remaining local anesthetics with reference to their synergism with epinephrin and have found that it is present, though not always as marked as with cocain and procain. The synergism is often absent when the doses of the cocain substitutes are sufficient to cause even a slight initial fall of blood pressure, but when they are so small as to produce no appreciable effect, when given alone, the synergism with epinephrin is quite definite. It is not very marked with alypin and apothesin. The mechanism of this peculiar synergistic action is not understood and we hope to make it the subject of a more detailed investigation.

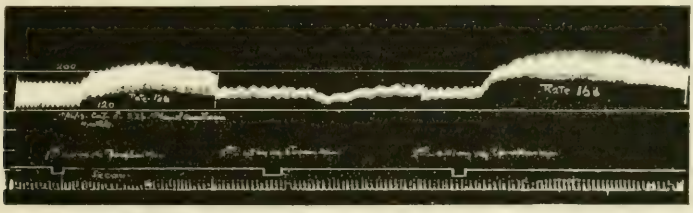


FIG. 6

Figure 6 illustrates this synergism, and it will be noted that the rise produced by a fixed dose of epinephrin is not only greater, but is also more sustained after the administration of the anesthetic than before, a condition precisely analogous to that shown in the case of procain.

11. Measures for resuscitation

(*a.*) *Artificial respiration, with or without cardiac massage.* In the search for methods of resuscitation we thought first of employing artificial respiration, or cardiac massage, or both together. It was apparent that if these measures should prove effective they should be capable of preventing death in the cat after the injection of appreciably more than the average fatal dose or they would be of relatively little value in cases of intoxi-

cation in man. We had two reasons for adopting this view; first, because in cases of accidental poisoning in man the amount of drug absorbed cannot be determined and it may often be well above the minimum which would be fatal in a given individual. Second, because the rapid destruction of the anesthetics in the liver should promptly reduce the amount of the drug free to act upon the heart and respiratory center to a point below that which would prove fatal, provided these two could be kept functionating for a few minutes.

Our method of procedure for determining the value of artificial respiration alone, or with cardiac massage, was to insert a tracheal cannula through a small incision after anesthetization of the skin with phenol, and to connect this with a respiration valve which was connected with an artificially interrupted air supply. We were thus ready to begin artificial respiration the moment that spontaneous breathing stopped, and could carry it on efficiently and uniformly for as long as might be desired. The drug to be used was injected rapidly into the femoral vein. When cardiac massage was employed it was given manually by rapid rhythmic compression and relaxation of the soft thorax over the heart, and proved to be adequate to maintain a fair blood pressure even after the heart had stopped (see tracings). We performed a series of eighteen experiments, the important points of which are shown in table 5. In every instance the attempts to resuscitate were continued until we were certain that the animal would not recover, generally for at least ten minutes.

The results of this series of experiments show that neither artificial respiration alone, nor combined with cardiac massage, is an efficient method for the resuscitation of cats after doses of the local anesthetics varying between 117 and 200 per cent of the fatal. In only two of the eighteen experiments did the cat recover, and in neither was cardiac massage employed, the heart resuming spontaneously after brief temporary cessation. It would seem probable that these two cats were naturally more tolerant to the local anesthetics than general, such tolerance having been noted occasionally.

TABLE 5

Showing results of applying artificial respiration and cardiac massage on recovery from large doses of local anesthetics

ANESTHETIC	DOSE		RESUSCITATIVE MEASURE	RESULT
	Mgm. x kgm.	Per cent of fatal		
Alypin	12.5	125	Artificial respiration	Death
Apothesin	25.0	125	Artificial respiration	Recovery
	40.0	200	Artificial respiration	Death*
Beta-eucain	15.6	125	Artificial respiration	Death
	18.7	150	Artificial respiration; cardiac massage	Death
Cocain	20.0	133	Artificial respiration; cardiac massage	Death
Holocain	12.5	125	Artificial respiration	Death
	12.5	125	Artificial respiration; cardiac massage	Death
Nirvanin	37.5	117	Artificial respiration	Death
	37.5	117	Artificial respiration; cardiac massage	Death
Procain	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
Stovain	37.5	135	Artificial respiration	Death
	37.5	135	Artificial respiration; cardiac massage	Death
Tropacocain	25.0	125	Artificial respiration	Recovery

* This was the same animal which received the dose of 25 mgm. x kgm. of apothesin one hour earlier.

(*b*) *Influence of epinephrin plus artificial respiration and cardiac massage.* We stated in our earlier paper on procain that the simultaneous intravenous injection of epinephrin interfered to some extent with the toxic action of procain, and we there recorded one instance of a cat's surviving a dose of procain 50 per cent larger than that usually fatal when it was injected simul-

taneously with epinephrin. Those observations suggested the further study of the value of epinephrin as an aid to resuscitation, and we performed a series of seventeen experiments which are illustrated by the following representative protocol and accompanying tracing—figure 7.

Protocol 18. Cat, male, 3.34 kgm.

Skin anesthetized with phenol and connection made with tracheal cannula for artificial respiration. Cannula into left femoral vein for injection of epinephrin in 1–100,000 solution. Normal tracing.

10.30 a.m. 18.7 mgm. x kgm. beta-eucain into right femoral vein. Prompt cessation of respiration and heart, blood pressure fell to zero. Artificial respiration and injection of epinephrin begun at once. Cardiac massage twice to carry epinephrin to heart. Prompt recovery of heart; artificial respiration stopped after about three minutes. Complete recovery.

11.30 a.m. 12.5 mgm. x kgm. beta-eucain as before. No attempt to resuscitate. Typical death, except for temporary partial recovery of heart.

The importance of carrying on artificial respiration in addition to the injection of epinephrin is illustrated by another experiment, the details of which are shown on the graphic record—figure 8. This experiment shows the beginning recovery of the heart about two and one half minutes after a dose of apothesin twice that usually fatal. The heart then improved rapidly and the blood pressure rose above the level previous to injection. Cessation of artificial respiration was followed by a rapid fall in the blood pressure with weakening of the heart. Reinstitution of artificial respiration rapidly restored the heart. This phenomenon was repeated once more, after which recovery was complete. Neither of the two temporary interruptions of artificial respiration was followed by the resumption of spontaneous breathing. This was probably the result of the direct action on the respiratory center of the very large dose of apothesin, and was the probable cause of the beginning failure of the heart on both occasions.

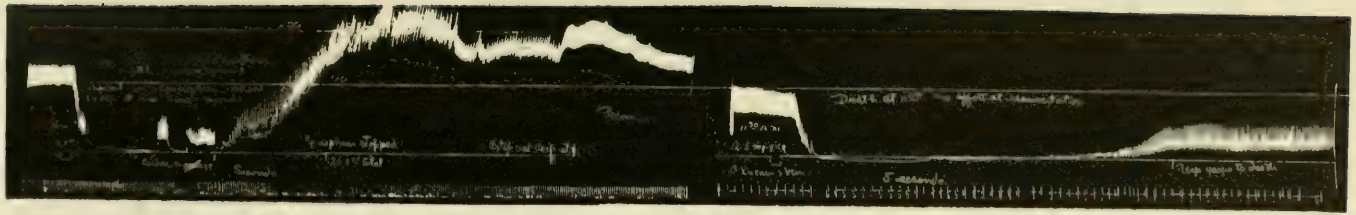


FIG. 7

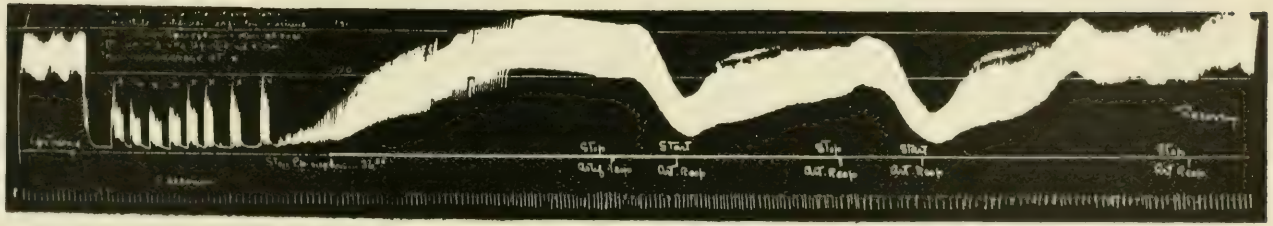


FIG. 8

TABLE 6

Showing effects on toxicity of large doses of the local anesthetics from the intravenous injection of epinephrin combined with artificial respiration and cardiac massage

ANESTHETIC	DOSE		EPINEPHRIN 1-100,000 cc. x kgm.	ARTIFICIAL RESPIRATION	CARDIAC MASSAGE	RESULT
	Mgm. x kgm.	Per cent of fatal				
Alypin	12.5	125	7.7	+	+	Recovery
Apothesin	25.0	125	7.5	+	0	Recovery
	30.0	150	9.3	+	+	Recovery
	40.0	200	13.0	+	+	Recovery
Beta-eucain	18.7	150	6.0	+	+	Recovery
Cocain	20.0	133	8.0	+	0	Recovery
	22.5	150	8.1	+	+	Death
Holocain	12.5	125	9.4	+	0	Death*
Nirvanin	37.5	117	5.5	+	+	Recovery
Procain	50.0	120	8.2	+	+	Death
	50.0	120	11.7	+	+	Recovery†
	60.0	142	8.0	+	+	Recovery
	80.0	190	9.0	+	+	Death‡
Stovain	37.5	135	6.1	+	+	Death
	37.5	135	2.5	+	+	Recovery
Tropacocain	25.0	125	9.9	+	+	Recovery
	30.0	150	2.2	+	+	Death

* Recovery was apparently complete and resuscitative measures were stopped. Heart and respiration failed together five minutes later.

† This cat had received the dose of apothesin of 30 mgm. x kgm., recorded above, half an hour previously.

‡ Recovery was apparently complete in ten minutes and all resuscitative measures were stopped fifteen minutes after injection of the anesthetic. Spontaneous respiration was not resumed and heart began to fail after about one minute, but was restored promptly by reinstitution of artificial respiration. This was continued for nine minutes when spontaneous breathing began. Nine minutes later the animal died, the respiration failing before the heart. The cat in this experiment had received the dose of 60 mgm. x kgm. of procain, recorded above, only twenty-two minutes earlier.

The chief points in the several experiments in this series are shown in table 6, from which it is evident that the combination of the intravenous injection of epinephrin with effective artificial respiration, with or without resort to cardiac massage, is usually capable of saving the cat from doses of the local anesthetics varying from 117 to 200 per cent of the average fatal. Death resulted in only six of the seventeen experiments, and in one of these it followed the administration of nearly twice the fatal dose of the anesthetic—procain—to a cat which had received almost one and a half times the usual fatal dose of the same drug only twenty-two minutes previously. Control experiments showed that normal saline was without value in antagonizing the local anesthetics.

While cardiac massage cannot be carried out so efficiently in man as in the cat, it can be performed sufficiently to cause the greater part of an intravenous injection of epinephrin to reach the heart; and several satisfactory methods are available for carrying on artificial respiration in man. These measures, also, are all such as can be applied without loss of time under most conditions and they certainly seem to be the most effective means at our disposal for saving life after the development of symptoms of acute intoxication by the anesthetics of this group. Since acute poisoning in man is accidental and occurs when least expected, those who frequently employ these local anesthetics should always be prepared to apply the three resuscitative measures of intravenous infusion of epinephrin, artificial respiration and cardiac massage, and all three should be used in combination since no one alone is effective except in rare instances.

12. Antagonism by ouabain

The fact that the several local anesthetics promptly bring the cat's heart to standstill has been shown to be characteristic of their acute toxic action, but this paralysis, while immediate, is not permanent in all cases, even after fatal doses, for we not infrequently observe a temporary, though rather feeble, spontaneous return of the heart beat, as illustrated in figure 3. These

facts, combined with the rapid destruction of the anesthetics by the liver, prompted us to try the effect upon their toxicity of the previous stimulation of the heart by one of the digitalis bodies. For the sake of convenience we selected ouabain, and also because it has a fairly well fixed fatal dose upon which we could base our calculations. We administered 60 per cent of the fatal dose of ouabain to several cats, either intravenously or intramuscularly, and after intervals of fifteen minutes to several hours, gave more than an average fatal dose of one of the local anesthetics in the usual manner. Artificial respiration was employed in most cases to tide over the period required for recovery of the heart, for we found that without it the cat would die from the respiratory paralysis even when the heart was evidently recovering. Our first four experiments were made with procain and we present an abbreviated protocol to illustrate the results.

Protocol 19. Cat, male, 2.94 kgm.

9.51 a.m. 0.06 mgm. x kgm. ouabain intravenously.

10.21 a.m. 60.00 mgm. x kgm. procain intravenously in 20 per cent solution. Prompt convulsion; respiration stopped in about one minute; heart feeble, but continued to beat. Artificial respiration started at once and continued for five minutes, during which the heart rapidly recovered. Complete recovery within fifteen minutes.

Although these first experiments were controlled by the administration of the same dose of procain to two normal cats with prompt death, in spite of artificial respiration, the results were so striking that we undertook a more rigid control. For this purpose one of us injected four cats with either normal saline, or with 0.06 mgm. x kgm. of ouabain, and the other then administered 60 mgm. x kgm. of procain to each, carrying out artificial respiration in all, and was able to identify correctly the two cats which had received ouabain by the fact that they recovered while the other two died promptly.

TABLE 7

Showing effect on toxicity of the local anesthetics of the previous administration of ouabain, supplemented by the use of artificial respiration

ANESTHETIC	DOSE		DOSE OF OUABAIN		ARTIFICIAL RESPIRATION	RESULT
	Mgm. x kgm.	Per cent of fatal	Mgm. x kgm.	Per cent of fatal		
Apothesin	30.0	150	0.01	10	+	Death
	30.0	150	0.02	20	+	Death
	30.0	150	0.06	60	0	Recovery*
	40.0	200			+	Death†
	35.0	175	0.06	60	+	Recovery
Beta-eucain	18.7	150	0.06	60	+	Recovery*
Cocain	20.0	133	0.06	60	+	Death‡
	20.0	133	0.06	60	+	Recovery*
Holocain	12.5	125	0.06	60	0	Recovery*
Nirvanin	37.5	117	0.01	10	+	Death‡
	37.5	117	0.02	20	0	Recovery*
	40.0	125	0.06	60	0	Recovery*
Procain	60.0	142	0.02	20	+	Recovery
	60.0	142	0.06	60	0	Death
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery§
	60.0	142	0.06	60	+	Recovery§
Stovain	37.5	135	0.02	20	+	Recovery
	37.5	135	0.06	60	+	Recovery
	40.0	148	0.06	60	+	Death‡¶
Tropacocain	25.0	125	0.05	50	+	Recovery

* In the experiments thus marked the heart did not stop.

† This animal had received the preceding dose of 30 mgm. x kgm. of apothesin 55 minutes earlier.

‡ In the experiments thus marked cardiac massage was employed.

§ In these two experiments the cats were identified as having received ouabain, unknown to the experimenter—see text.

¶ This animal had been given the dose of 40 mgm. x kgm. of nirvanin, recorded above, one hour earlier.

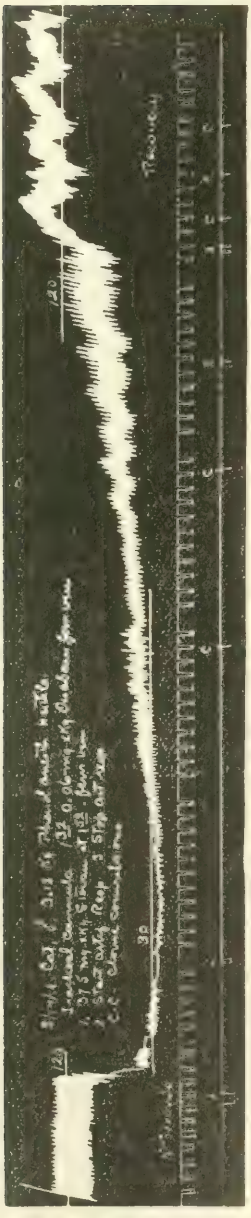


Fig. 9

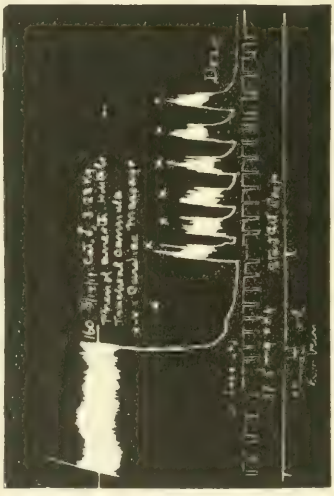


Fig. 10

Table 7 gives the important details of each of this series of twenty-three experiments and shows that the administration of a dose of 0.02 mgm. x kgm. or over of ouabain effectively protects the heart from permanent stoppage by doses of the local anesthetics ranging from 117 to 175 per cent of the average fatal. Of the twenty-one experiments in which 0.02 mgm. x kgm. or more of ouabain was given, death followed the administration of the local anesthetic in only five. Of these five deaths, one followed the administration of twice the fatal dose of apothesin to a cat which, fifty-five minutes before, had been given a dose of the same drug 50 per cent greater than the average fatal and had recovered. A second death followed the administration of 148 per cent of the fatal dose of stovain to a cat which had survived 125 per cent of the average fatal dose of nirvanin given an hour earlier. Excluding these two, death followed the administration of these large doses of the local anesthetics, given after the previous administration of 20 to 60 per cent of the fatal dose of ouabain, in only three out of nineteen cats. Artificial respiration was employed in all but five of the experiments, in four of which it was not necessary, while in one it was omitted purposely. Cardiac massage was tried in three of the experiments in which the heart did not show evidences of recovering, but it was without effect. By way of graphic illustration of this antagonism we introduce figures 9 and 10, taken from two parallel experiments.

DISCUSSION

One fact of fundamental importance has emerged from the investigations detailed in the preceding pages; namely, that the "essential" elimination of all of the local anesthetics studied, excepting cocain and holocain, proceeds with great rapidity and is completed within a few minutes following the intravenous injection of a sublethal dose. The "essential" elimination of cocain and holocain is a much slower process, and in the case of cocain it may not be complete after periods of one to two, or more, days. The elimination of all of the local anesthetics in the cat has been shown to be accomplished by their destruction

in the liver, as demonstrated by perfusion of that organ with solutions of the several drugs. In view of the close similarity of his behavior to toxic doses of the local anesthetics, it is highly probable that man also eliminates these drugs in the same way as the cat, especially since such rapid elimination by the kidney is most unlikely.

The local anesthetics can be divided into two groups according to their rates of destruction or "essential" elimination in the cat: Group 1, rapidly eliminated, includes alypin, apothesin, beta-eucain, nirvanin, procain, stovain and tropacocain. Group 2, slowly eliminated, includes cocain and holocain. This grouping apparently applies also for man.

The prompt recovery of the cat following the intravenous injection of a just sublethal dose of any of the members of group 1 is due to the rapid destruction of those drugs, while slower destruction in the case of cocain and of holocain explains the less rapid and less complete recovery after corresponding doses.

The ability of the cat to withstand repeated intravenous injections of large fractions of the minimal fatal vein doses of members of group 1, when given at intervals of fifteen to twenty minutes, or to survive the slow and continuous injection of several times the average fatal vein dose, depends upon the rapid "essential" elimination of those drugs, and the inability to withstand similar injections of corresponding amounts of the drugs of group 2 is due to their slower elimination.

Rapid "essential" elimination of the members of group 1 accounts for their relatively low toxicity by subcutaneous injection, or after their application to mucous membranes, in the cat and other animals, in spite of the fact that they are absorbed rapidly from the subcutaneous tissues and from some mucous surfaces. It also explains the marked reduction in the toxicity of these drugs effected by the delayed absorption produced by the simultaneous injection of epinephrin. Slower "essential" elimination of the members of group 2 accounts for their relatively higher toxicity after subcutaneous injection, or application to mucous membranes, and for the lesser reduction in their toxicity effected by epinephrin.

Man also apparently recovers more rapidly from non-fatal acute poisoning by the members of group 1 than from cocain, the only member of group 2 on which we have data, and the difference is almost certainly again referable to his ability to destroy the drugs of the first group more rapidly and completely than he can cocain.

Finally, the successful resuscitation of cats, after intravenous doses of 117 to 200 per cent of the average fatal, by means of artificial respiration and stimulation of the heart by epinephrin, and the protection of cats from death, following doses of 117 to 175 per cent of the average fatal, by the previous stimulation of the heart by ouabain and the brief subsequent employment of artificial respiration, are both due primarily to the rapid destruction of the local anesthetics, by which the excess administered is promptly eliminated.

SUMMARY

1. The more important literature of acute intoxication in man from the local anesthetics is reviewed, and cases are cited to show the close similarity between the symptoms produced in man and in the lower animals, especially the cat.

2. The phenomena of acute intoxication in the cat, following the intravenous injection of the local anesthetics, are described, and the similarity of the phenomena produced by different members of the group is emphasized.

3. The maximum toxicity for each of the members of the series has been determined by rapid intravenous injection in the cat, and the relative toxicities of the several drugs are represented graphically.

4. The several different local anesthetics are shown to be mutually and quantitatively synergistic, so far as their fatal actions are concerned.

5. The capacity of the cat to withstand the intravenous injection of several times the fatal vein dose of any of the local anesthetics, except cocain and holocain, has been shown by repeated injections of large doses, or the continuous injection of relatively dilute solutions.

6. The toxicity of the local anesthetics for the cat, after subcutaneous injection, has been shown to depend upon the ratio between the rate of absorption and that of elimination, and the local anesthetics can be divided into two classes with reference to that ratio. Five, or more than five, times the minimal fatal vein dose of alypin, apothesin, beta-eucain, nirvanin, procain, stovain and tropacocain can be injected subcutaneously in the cat without causing death, while four, or less than four, times the fatal vein doses of cocain and holocain similarly injected prove fatal.

7. The simultaneous subcutaneous injection of epinephrin with the local anesthetics materially reduces the toxicity of the latter by delay in the rate of their absorption, but this reduction is much less marked in the cases of cocain and holocain than with the other members of the series, and is referable to their much slower "essential" elimination.

8. The absorption of several of the local anesthetics from the mucous membranes of the nose and pharynx of the cat has been shown to be no more rapid than from the subcutaneous tissues, and the urethra, bladder and vagina resist the absorption of these drugs to a great extent.

9. The elimination of the local anesthetics in the cat has been demonstrated to be due to their rapid destruction by the liver, and this takes place in the excised, perfused organ as well as in the liver of the intact animal.

10. Various efforts have been made to influence the toxicity of the local anesthetics for the cat, and severe acute hemorrhage and narcosis by hydrated chloral alone seem to have any material influence. Both of these measures tend to increase the cat's susceptibility to the toxic actions of the local anesthetics, probably by diminishing the rate of their destruction in the liver through impairment of the circulation.

11. All of the local anesthetics have been shown to be synergistic with epinephrin on the blood pressure in a manner analogous to cocain.

12. The employment of artificial respiration, combined with stimulation of the heart by the intravenous injection of epi-

nephrin, is capable of saving cats from death following the intravenous injection of as much as twice the average fatal dose of the local anesthetics.

13. Stimulation of the heart by the previous injection of ouabain permits the cat to recover from intravenous injections of nearly twice the average fatal dose of the local anesthetics, when the temporary paralysis of the respiratory center is combated by the use of artificial respiration.

14. The success of the last two measures depends upon the rapid destruction of the local anesthetics by the liver.

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